SOCIETY FOR NEUROSCIENCE

ABSTRACTS

VOLUME 15, PART 2

19TH ANNUAL MEETING
PHOENIX, ARIZONA
OCTOBER 29–NOVEMBER 3, 1989

Made in the United States of America.
International Standard Book Numbers:
Part 1 ISBN 0–916110–31–1
Part 2 ISBN 0–916110–32–X
Both parts ISSN 0190–5295
Library of Congress Catalog Card Number 75-7761

Proper citation form for this volume:
Soc. Neurosci. Abstr., Vol. 15, Part 2, p. xxx, 1989.

Published by:
Society for Neuroscience
11 Dupont Circle, N.W.
Suite 500
Washington, D.C. 20036

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*8,138 volunteer abstracts, 17 symposia.

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TUESDAY

FRIDAY

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Session Number	Session Title	Type	Day and Time

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245.	Cerebellum III	Poster	Tue PM
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344.	Alzheimer's disease: transmitters and behavior	Poster	Wed PM

387.9

A LIGAND-GATED CHANNEL RECEPTOR FOR HISTAMINE. T. S. McClintock and B. W. Ache*. Whitney Lab. and Depts. of Zool. and Neurosci., Univ. of Florida, St. Augustine, FL 32086.

Histamine (HA) suppresses spiking when applied to the soma of lobster olfactory receptor neurons. In voltage-clamped somata, HA activates a Cl^- conductance. The Cl^- current was unaffected by external Co^{++} and Cd^{++}, or internal perfusion with activators and inhibitors of G-proteins. HA activated a Cl^- channel when pulsed onto outside-out (N = 35) but not cell-attached patches (N = 19). Intracellular GTP-gamma-S did not affect channel gating by HA. These data demonstrate that HA directly gates a Cl^- channel with a conductance of 66 pS in the Caribbean spiny lobster and 44 pS in the American lobster. The percent of time the channel spent in the open state (P_o) was a graded function of dose, beginning between 0.1 and 1 μM HA. Some kinetic properties of the channel were sensitive to voltage. The macroscopic current decayed rapidly as a single exponential (τ of about 100 ms) at -70 mV, as a double exponential between -60 and -35 mV (τ's of about 100 and 1000 ms), and did not decay above -35 mV. Similarly, the P_o of the channel was increased 10- to 15-fold when the potential was increased from -50 mV to 50 mV. This new ligand-gated channel shares some properties with the $GABA_A$ receptor.

Supported by the Grass Foundation, NIMH award F31MH09495 and NSF award BNS88-10261.

387.10

SOME PROPERTIES OF 5-HT_3 RECEPTOR CHANNELS: SINGLE CHANNEL AND WHOLE CELL RECORDINGS FROM SUBMUCOSAL NEURONS. A.Surprenant, V.A.Derkach*,K-Z.Shen* & R.A.North. Vollum Institute, Oregon Hlth.Sci.Uni., Portland, OR 97201

Results have accumulated to suggest that 5-HT_3 receptors are ligand-gated ion channels. We sought to provide direct evidence for such speculation by recording currents in response to 5-HT application from outside-out membrane patches obtained from dissociated guinea-pig submucosal neurones. External/internal solutions were:NaCl 164,$MgCl_2$ 1, $CaCl_2$ 1, glucose 11, HEPES 10/Cs-glutamate 160, $MgCl_2$ 1, CaCl 1, EGTA 10, HEPES 10. 5-HT (1-10 μM) caused the appearance of unitary inward currents of two discrete amplitudes whose chord conductances were 15 pS and 9 pS. Open probability (p_o) of the smaller, but not the larger, channel fell markedly during 2-5 min application of 5-HT; therefore we studied mainly the 15 pS channel. This channel showed fast openings and bursts of openings; uncorrected open times showed two exponentials (τ_1 = 0.4 ms, τ_2 = 6 ms) and at least three closed states were demonstrated. ICS 205-930, reduced p_o, primarily by altering the closed states.

Whole cell recordings (using identical solutions) were made to further characterize the 5-HT_3 response. 5-HT (1-10 μM) caused rapidly desensitizing inward currents which reversed at +3 mV with a $Na^+/K+$ conductance ratio of 2.3; the response did not require internal ATP or GTP and was not blocked by pertussis toxin pretreatment.

387.11

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

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

ATP elicited an outward potassium current at the reversal potential for the early excitatory current. Activation of the potassium current was calcium independent, since the magnitude of the current was unaffected by dialyzing cells with 20 mM BAPTA and no calcium. Activation was also independent of G protein alpha subunits, since the current was similar when cells were dialyzed for 10-15 minutes with either GTP, GDP-beta-S, or GTP-gamma-S. Activation of the current through protein phosphorylation was unlikely, since dialysis with an intracellular cocktail of metabolic inhibitors had no effect. Noise analysis yielded spectral estimates for the single channel conductance of approximately 20 pS. Activation of the potassium current by cytoplasmic second messengers was ruled out when we found that ATP activated 22 pS potassium channels in outside-out membrane patches. If a second messenger is involved, these results suggest that it is intramembranous. Possible candidates include arachidonic acid, released from lipids by phospholipase A_2, and its metabolites, prostaglandins and leukotrienes. We have found that several prostaglandins activate potassium channels in outside-out membrane patches, and are currently examining whether these channels are the same as those activated by ATP.

387.12

CATIONS AND ANIONS PERMEATE A SINGLE ATP-ACTIVATED CHANNEL IN CHICK SKELETAL MUSCLE. R. I. Hume and S. A. Thomas. Department of Biology, University of Michigan, Ann Arbor, MI 48109

Micromolar concentrations of extracellular adenosine 5'-triphosphate (ATP) elicit a rapid excitatory response in developing chick skeletal muscle. Excitation is the result of a simultaneous increase in membrane permeability to cations and anions. ATP does not induce the formation of large membrane pores, since ions such as tetraethylammonium and glucuronate are impermeant during the response. Here we test whether cations and anions permeate a single ATP-activated channel which does not discriminate by charge, or whether they permeate separate cation and anion channels concurrently activated by ATP.

Experiments were performed on myoballs using the whole-cell patch-clamp technique. This method permitted internal dialysis of the cells and control of the membrane potential. In order to determine if one or two types of ATP-activated channels were present, we analyzed the fluctuations about the mean current induced by ATP. Ionic conditions were arranged so that the reversal potential for cations was +50 mV and the reversal potential for anions was -50 mV. Under these conditions, if ATP activates a single channel that does not select by charge, ATP should not evoke an increase in noise at its reversal potential. However, if ATP activates separate cation and anion channels, ATP should evoke a significant increase in noise at its reversal potential. At both +40 mV and -50 mV ATP elicited a clear increase in noise, but at the ATP reversal potential of -5 mV no increase in noise above background was seen. These results indicate that there is only a single excitatory ATP-activated channel type, which is nonselective by charge. Based on analysis of the noise spectrum, the conductance of the channel was approximately 0.3 pS.

PEPTIDES: PHYSIOLOGICAL EFFECTS III

388.1

ROLES OF SOMATOSTATIN (SRIF) AND GROWTH HORMONE RELEASING FACTOR (GRF) IN ETHER STRESS INHIBITION OF GROWTH HORMONE (GH) RELEASE. M.C. Aguila*, R. Pickle*, W. Yu* and S.M. McCann. Dept. Physiology, Neuropeptide Div., Univ. TX Southwestern Med. Ctr., Dallas, Texas 75235.

In order to evaluate the release of somatostatin (SRIF) and growth hormone releasing factor (GRF) into the pituitary gland in response to ether stress, a push-pull-perifusion (PPP) technique has been used in freely moving rats. Push-pull cannulae (PPC) were implanted into the anterior pituitary (AP) gland of male rats (250-270 g). After a 7 day recovery period the rats were fitted with indwelling jugular catheters. The next day the animals were subjected to PPP of the AP during one hour followed by ether stress (2 min) and another hour of perifusion. The perifusion flow was 20 μl/min and 10 min fractions were collected and assayed for SRIF and GRF by RIA. Plasma GH levels were assayed every 10 min. At the end of the experiments, the accuracy of PPC tip placements was ascertained with a dissecting microscope. Under basal conditions SRIF and GRF output pulsed at 20-40 min intervals. SRIF and GRF output in the ten min period beginning with application of ether was increased two-fold ($p<0.005$ and $p<0.01$, respectively). Interestingly, the release of SRIF continued for an additional 10 min, whereas GRF output decreased and was almost undetectable. The release of both GRF and SRIF receded to basal values 20-30 min after stress. Plasma GH levels were significantly lowered 10 min after stress. Each of the 9 animals showed a restoration of pulsatile GH release to above basal levels within 20 to 30 min after stress. Our findings provide compelling evidence that somatostatin plays a prominent role in stress- induced inhibition of GH release in the rat by blocking the response to the transient elevation of GRF and continuing to suppress GH release for 20 min.

388.2

NEUROPEPTIDE Y STIMULATES GnRH RELEASE FROM SIMIAN HYPOTHALAMI. K.-Y.F. Pau*, A.H. Kaynard* and H.G. Spies. (SPON. Y.F. Chen) Oregon Regional Primate Research Ctr., Beaverton, OR 97006.

Feeding, cardiovascular, and reproductive functions are modified by neuropeptide Y (NPY) action. In the rat and rabbit, NPY alters the secretion of gonadotropin-releasing hormone (GnRH) and luteinizing hormone (LH), both *in vitro* and *in vivo*. We utilized simian (macaque and baboon) hypothalami in *in vitro* superfusion studies to examine the release of GnRH and ß-endorphin (ß-EP) from isolated fragments of the anterior hypothalamus (AH) and the mediobasal hypothalamus (MBH) in response to NPY treatment. Within 3 min after death, blocks of hypothalamic tissue in ice-cold Locke's medium were sectioned along the ventricular midline into left and right halves. The AH/MBH blocks were distinguished rostrally by the anterior commissure and caudally by the mamillary bodies; the AH and MBH were separated at the caudal end of the optic chiasm. These 4 fragments (left and right AH, left and right MBH) were placed into individual superfusion chambers submerged in a 40°C-water bath and were superfused with Hepes-buffered Locke's medium (pH 7.40). Samples (400μl) were collected at 10-min intervals into tubes containing 40μl of 1N acetic acid. Hypothalamic fragments received either 6 h of Locke's medium (controls) or 3 h of Locke's medium followed by 3 h of 80 nM of NPY in Locke's medium. GnRH and ß-EP in perfusate samples were measured by RIA and values were compared by two-way ANOVA followed by Newman-Keuls. GnRH levels rose within 20-30 min of NPY treatment ($p<0.01$) and elevated GnRH release by both the AH (n=12) and MBH (n=13) was sustained for the duration of NPY exposure. NPY caused no measurable change in ß-EP concentrations in perfusates of AH (n=10) or MBH (n=9). Exposure to Locke's medium alone for 6 h caused no increase ($p>0.05$) in GnRH or ß-EP release from AH (n=6) and MBH (n=7). These results suggest that NPY stimulates GnRH neurons via mechanisms that do not involve the release of ß-EP. Supported by RR-00163, HD-16631, HD-18185, HD-07044.

388.3

A NOVEL HYPOTHALAMIC NEUROPEPTIDE WITH 38 RESIDUES (PACAP38) STIMULATES ADENYLATE CYCLASE ACTIVITY IN PITUITARY CELLS, NEURONS AND ASTROCYTES. G. Katsuura*, R.R. Dahl*, A. Miyata* and A. Arimura (SPON: F. R. Domer), U.S.-Japan Biomedical Res. Labs, Tulane Univ. Hebert Center, Belle Chasse, LA 70037; and Depts. of Medicine & Anatomy, Tulane Univ. Sch. of Med., New Orleans, LA 70112.

We have recently isolated and characterized a novel hypothalamic neuropeptide with 38 residues from ovine hypothalamic tissues by monitoring adenylate cyclase activation in rat pituitary cell cultures during isolation. This peptide was named as PACAP38 (Pituitary Adenylate Cyclase Activating Peptide with 38 residues). It was found that PACAP38 had 68% homology with porcine VIP in the C-terminus region 1-28. We examined the effect of synthetic PACAP38 on intracellular accumulation in cultured rat pituitary cells, neurons and astrocytes in comparison with VIP. In anterior pituitary cell cultures, PACAP38 (10^{-9} M) increased cAMP 4-fold at 1 min after addition, as compared with control levels. Cyclic AMP increased by 15-fold at 10 min. Phosphoinositide hydrolysis in pituitary cells was not altered by PACAP38. A similar increment of intracellular cAMP was observed in cultured neurons with the peak occurring at 10 min after addition of the peptide. cAMP levels began decreasing after 10 min in both pituitary cells and neurons. On the other hand, an extraordinarily large increase in cAMP accumulation was demonstrated in cultured astrocytes after addition of PACAP38 (10^{-9} M). Cyclic AMP increased 30-fold at 1 min and 100-fold at 5 min after addition, and remained elevated during a 60 min incubation. It was interesting to note that VIP at the same concentration (10^{-9} M) failed to increase intracellular cAMP levels in pituitary cells, neurons, or astrocytes. Although distribution of PACAP38 in the brain remains to be elucidated, the possibility that various central actions which have been attributed to VIP may actually be regulated by PACAP38 cannot be excluded. (This study was supported by NIH grant DK09094.)

388.4

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

One source of input to the AMYG is derived from GnRH neurons in the septal area (Jennes, Brain Res. 404: 339, 1987). The present electrophysiological study was designed to identify AMYG neurons receiving septal input, to determine the influence of estrogen priming on the orthodromic response, and to test the effect of iontophoretically applied GnRH. Ovariectomized female rats, primed (n=10) or not (n=18) with estrogen (EB, 2ug 44 hrs prior to recording) were anesthetized with urethane (1.4g/kg) and prepared for conventional *in vivo* extracellular recording. A stimulating electrode was positioned in the medial septal area to orthodromically drive AMYG neurons. A multi-barrelled glass pipette was used to record single-cell activity in the AMYG and to eject GnRH. Orthodromic responses were analyzed by collecting peristimulus-time histograms. In both primed and unprimed animals, a large percentage of neurons were orthodromically responsive (57 of 103 in primed animals and 46 of 70 in unprimed animals). EB-priming significantly increased the number of orthodromic inhibitory responses. In the small number of GnRH-responsive neurons, no consistent relationship between the GnRH response and the orthodromic response was observed. However, in a few instances, GnRH modulated the orthodromic response; i.e., decreased the amount of orthodromic excitation or inhibition. Although the identity of the neurotransmitter(s) remains to be established, the results demonstrate a substantial, estrogen-sensitive input from the septal area to the amygdala. HD09988 and NIH TG 5T35HL 07483-08.

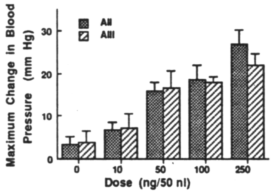
388.5

LHRH BLOCKS ACCOMMODATION OF CA1 PYRAMIDAL CELL DISCHARGE IN THE *in vitro* HIPPOCAMPUS SLICE PREPARATION. R. L. Moss, M. Wong*, and M. J. Eaton. Dept.of Physiology, Univ. Texas Southwestern Medical Center, Dallas, Tx 75235.

Specific luteinizing hormone releasing hormone (LHRH) receptor binding sites have recently been visualized with autoradiography in the hippocampus of the rat brain, in particular in the pyramidal cell layer (Br. Res. 452, 156, 1988). A previous study has suggested that application of LHRH produces a long lasting depolarizing response in CA1 hippocampal cells (Neuroend. 44, 137, 1986). The present series of experiments were designed to elucidate more specifically the effects of LHRH on the intracellular electrophysiology of CA1 neurons in the *in vitro* hippocampal slice preparation. Transverse vibratome sections (425 microns) through the hippocampus were obtained from female rats and subsequently superfused with oxygenated, normal artifical cerebrospinal fluid (ACSF) at a flow rate of 2.0 ml/min at 32°C. Single barrel glass electrodes were filled with either 3M KCl (80-90 MΩ) or 3M K acetate (90-120 MΩ) and utilized for intracellular recording as well as for applying depolarizing and hyperpolarizing stimuli to the cell via a Medical Systems DC Amplifier. The findings reported here are based on intracellular recordings from over 40 cells with resting membrane potentials of greater than -55mV. Superfusion of LHRH (10^{-7} and 10^{-9}M) initiated a short latency, long duration depolarization of CA1 neurons of the hippocampus that was accompanied by an increase in input resistance of the cell and was not affected by the application of a low Ca^{2+}, high Mg^{2+} ACSF. This result suggests that LHRH acts directly on the pyramidal cell membrane. We have also found that LHRH blocks or reduces the spike frequency adaptation (accommodation) which normally occurs with depolarizing stimuli. Research is presently in progress to examine the underlying mechanisms of LHRH action on hippocampal pyramidal cells. (NIH MH-44591 & HD-09988).

388.6

PARAVENTRICULAR MICROINFUSIONS OF AII AND AIII IN NORMOTENSIVE RATS INDUCE PRESSOR RESPONSES. Laurie L. Jensen, Joseph W. Harding, and John W. Wright. Washington State University,Pullman, WA.. 99163. Angiotensin II (AII) immunoreactivity has been reported in cells of the paraventricular nucleus of the hypothalamus (PVH); (Lind & Swanson, 1985), and the microiontophoretic applications of AII and angiotensin III (AIII) has been shown to excite PVH cells with AIII inducing significantly shorter latencies of activity than AII (Harding & Felix, 1987). This finding was interpretted to suggest that AII may have to undergo conversion to AIII in order to serve as a ligand. In the present experiment anesthetized rats received microinfusions of AII and AIII at doses of 0, 10, 50, 100, and 250 pmoles in a total volume of 50 nl aCSF into the PVH. The figure below indicates dose dependent increases in blood pressure to both AII and AIII, thus extending earlier findings by Brosnihan et al. (1987) who tested only AII, and supporting the notion that AIII is an important ligand in the PVH. Supported by NIH grant HL32063 & TW01112 and the American Heart Association.



388.7

DES-LEU ANGIOTENSIN I IN THE RENIN-ANGIOTENSIN PATHWAY — IS THERE A SECOND CARBOXYL CLEAVAGE? D. G. Changaris* and R. S. Levy, Depts. of Surgery & Biochemistry & Lab. Biol. Psychiatry, University of Louisville School of Medicine, Louisville, KY 40292

Des-leu angiotensin I (AI-dl) is twenty to thirty times more potent hypertensively when injected into the brain of the rat as opposed to injection into the cardiovascular system. This peptide is nearly equipotent with angiotensin II (AII) in its ability to induce the drinking response when injected into the cerebroventricles (Regul. Pept. 20, 273-280, 1988). Since both captopril and saralasin can inhibit this response, it is likely that the drinking response requires the hydrolysis of AI-dl to angiotensin II (AII) or to the heptapeptide, des-phe angiotensin II. To explore the first possibility, D-amino acid substitutions of the carboxyl terminus were made during the synthesis of AI and AI-dl. The dipsogenic responses from intraventricular (IVT) injections of doses ranging from 1.0 to 2.5 nmoles of each peptide were recorded. D-leu AI was half as potent as the normal L-substituted AI after IVT injections of 1.0 to 2.5 nmoles. D-his AI-dl produced a negligible drinking response at these doses as compared with the normal L-substituted AI-dl. These data support the observation that a second, single carboxyl cleavage of AI-dl occurs before it can act as a dipsogen in the rat. (Supported by NIH-CIDA 531621; VA-DOD 002; and Glenmore Foundation.)

388.8

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

We have recently demonstrated that the antipyretic action of indomethacin is dependent upon the vasopressin (AVP) V_1-receptor within the ventral septal area (VSA) of the brain. The purpose of this study was to assess the antipyretic effects of sodium salicylate (SALIC) and acetaminophen (ACETA) during V_1-receptor blockade within the VSA. Male SD rats were stereotaxically implanted with VSA and intracerebroventricular (icv) cannulae. Body temperature (T_b) was monitored remotely via radiotelemetry. Fever was induced by E.coli endotoxin icv. After 150 min the V_1-antagonist or saline was administered into the VSA immediately followed by SALIC or ACETA (75 mg/kg) ip. A 2h thermal index was calculated from the time of drug administration for statistical analysis. The V_1-antagonist attenuated SALIC-induced antipyresis in a dose-related manner: saline(VSA)+SALIC, -1.71 ± 0.20 °C hr; 1.0 µg V_1-antagonist(VSA)+SALIC, -0.57 ± 0.13 °C h ($p<0.05$); 10.0 µg V_1-antagonist(VSA)+SALIC, $+0.06\pm0.48$ °C h ($p<0.01$). However, the antipyresis induced by ACETA was unaffected by the V_1-antagonist. Neither dose of the V_1-antagonist or saline within the VSA affected non-febrile T_b. These results indicate that, like indomethacin, SALIC but not ACETA activate AVP V_1-receptors within the VSA during drug-induced antipyresis. (Supported by the MRC of Canada.)

388.9

BRADYKININ EVOKES AN INWARD CURRENT IN A SENSORY NEURON-DERIVED CELL LINE. John N. Wood, Philip M. Dunn*, Pat Hogan+, and Humphrey P. Rang* (SPON. L. Lillien) The Sandoz Institute, 5 Gower Place, London W.C.1, +Department of Neurobiology, Harvard Medical School, Boston, Mass 02115.

A battery of DRG-derived cell lines (ND cells) has been generated by fusing the HAT-sensitive neuroblastoma line N18Tg2 with neonatal rat DRG cells. After sub-cloning, lines that expressed the rat surface marker Thy 1.1, and the sensory neuron marker globoseries and lactoseries glycolipids were investigated. The sensory neuron-like characteristics exhibited by various lines include expression of δ-opioid receptors, depolarisation in response to capsaicin and susceptibility to latent infection with Herpes Simplex Virus. One line - ND7/23 - when differentiated with 1mM dibutyryl cAMP in the presence of 0.5% FCS and NGF, responded to bradykinin with an inward current and an increase in membrane conductance (18/50 cells). The mean latency of the response was 7.5 ±1.6 sec, the time to peak 47 ±6.8 sec, and the amplitude of the response was 0.2 ±0.05nA. The reversal potential (n=3) was 11.6 ±6mv. The characteristics of this response are shared by DRG neurons, and are distinct from the hyperpolarisation with an outward current demonstrated by other neuronal cell lines (e.g.NG108.15). ND7/23 cells, like DRG neurons, show elevated levels of cGMP on bradykinin application. Injection of mRNA from rat DRG and ND7 cells confers bradykinin sensitivity on Xenopus oocytes, which depolarise with the characteristics of an IP3-coupled response on bradykinin application. This cell line thus provides a useful model for the analysis of bradykinin-evoked activation of sensory neurons.

388.10

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

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

388.11

NEUROANTIBODIES: MOLECULAR CLONING OF A MONOCLONAL ANTIBODY AGAINST SUBSTANCE P FOR ITS EXPRESSION IN CELLS OF THE NERVOUS SYSTEM. A.Cattaneo*, F.Ruberti* and P.Piccioli* (SPON: F.Tirone). CNR Institute of Neurobiology, Roma (Italy).

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

388.12

FUNCTIONAL SIGNIFICANCE OF CHOLINERGIC AND VIP-ERGIC EFFECTS AT THE THYROID GLAND. L.J. Huffman*, M. Michalkiewicz*, J.M. Connors*, Z. Pietrzyk* and G.A. Hedge, Department of Physiology, West Virginia University Health Sciences Center, Morgantown, WV 26506.

In the thyroid gland, vasoactive intestinal peptide (VIP) and acetylcholine (ACh) are found in nerve fibers associated with secretory cells and blood vessels. We have, therefore, initiated studies to explore the actions and interactions of ACh and VIP in the regulation of thyroid blood flow (BF) and circulating hormone levels. Previously, we have shown that VIP increases thyroid BF in a dose-related manner. In order to evaluate whether VIP might exert any of its thyroidal effects via muscarinic receptors, we assessed the effects of ACh and VIP in the presence and absence of the muscarinic receptor blocker, atropine. Anesthetized male rats were treated iv with saline or atropine (3 mg/kg) 20 min before iv infusions of vehicle, ACh ($3x10^{-8}$ moles/100gBW), or VIP (10^{-11} moles/100gBW). Organ BFs were measured during this time using radiolabelled (^{141}Ce) microspheres. Mean systemic arterial pressure (MABP) was monitored and used in the calculation of organ vascular conductances (VC=BF/MABP). Atropine pretreatment tended to increase thyroid VC (170.7±37.3 vs. 104.8±19.7 μl/mmHg · min · g) and the vasodilatory effect of VIP was greater if the rats were pretreated with atropine. When the data were normalized for this stimulatory effect of atropine, the ACh-induced increase in thyroid VC was abolished during muscarinic blockade whereas the vasodilatory effect of VIP was unaffected. These results are consistent with the hypothesis that VIP and ACh exert their effects at the thyroid gland through independent mechanisms and that VIP release may be under prejunctional control via muscarinic receptors. (AM 35037)

388.13

ACETYLCHOLINESTERASE MODULATES MEMBRANE PROPERTIES AND SYNAPTIC TRANSMISSION IN MAMMALIAN CEREBELLAR CORTEX. H. Jahnsen* & M. Appleyard* (SPON: J. Mogensen). Institute of Neurophysiology, Blegdamsvej 3c, DK-2200 Copenhagen N, Denmark.

In addition to being a hydrolytic enzyme in the CNS acetylcholinesterase (AChE) may change membrane properties of central neurons. Indeed, in the substantia nigra AChE hyperpolarizes pars compacta cells thus changing their firing behavior (Greenfield et al., *Exp. Brain Res.*, 70:411, 1988). AChE is present in the cerebellar cortex, and can be released in a Ca^{2+} dependent manner by stimulation of climbing fibers (Appleyard et al. *Neurosci.*, 25:133, 1988). In order to measure possible effects on cerebellar neurons slices of guinea pig cerebella were cut and maintained using standard techniques. Intracellular recordings from Purkinje neurons revealed three different actions of AChE when added to the superfusing medium (10-20 U/ml). 1: The late part of the climbing fiber response was enhanced so that action potentials often was generated for tens of milliseconds after stimulation; 2: Responses to excitatory amino acids were increased in amplitude as well as in duration and 3: The threshold for firing of Ca^{2+} spikes in response to depolarizing intracellular current injection was increased. All the effects persisted when the catalytic site of AChE was blocked. These results indicate that AChE may have a role as modulator in the cerebellar cortex. An alternative possibility is that the enzyme has a tertiary structure similar to a natural endogenous modulator.

389.1

CYCLOPENTYLADENOSINE-INDUCED DOWNREGULATION OF A_1 ADENOSINE RECEPTORS IN EMBRYONIC CHICK HEART. T.A. Blair* and T.F. Murray (SPON:R.A.Dodson). Oregon State Univ., Corvallis, Oregon 97331.

Investigations utilizing embryonic chick atria had indicated that adenosine analogs elicit their negative chronotropic effect via an A_1 adenosine receptor (AdR). In the present study we have employed the developing chick heart as a model system to investigate the regulation of AdRs. Sustained activation of AdRs was accomplished with in ovo (day 9) injections of the A_1-selective adenosine agonist cyclopentyladenosine (CPA). Treatment with CPA resulted in a decreased AdR number as measured by the specific binding of the antagonist radioligand 8-cyclopentyl-1,3-[^{3}H]dipropylxanthine ([^{3}H]DPCPX). The binding parameters derived from nonlinear regression analysis of [^{3}H]DPCPX saturation isotherms indicated that in ovo treatment with 1 μmol CPA produced a 46$\pm$11% reduction in the density of A_1 receptors in cardiac membranes. The Kd value for [^{3}H]DPCPX binding to AdRs in CPA treated membranes was not significantly different from the saline treated value (CPA 2.9$\pm$0.1nM; saline 3.1$\pm$0.9nM). The decrease in receptor number was dose dependent suggesting a receptor mediated event. The maximum dose of CPA (10 μmol) resulted in a 77% decrease in the density of adenosine receptors with an ED_{50} for the CPA-induced down regulation of 2.5 μmol. Administration in ovo of 1 μmol CPA resulted in a decrease in the number of [^{3}H]DPCPX binding sites which was apparent by 4 hours and increased throughout the time span evaluated (24hr) when compared to saline treated controls. Co-injection of theophylline was able to attenuate the decrease in AdR number induced by 1 μmol CPA. These data suggest chronic exposure of embryonic day 9 chick hearts to an adenosine analog effects a downregulation of A_1 adenosine receptors.

389.2

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

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

389.3

EVIDENCE AGAINST THE INVOLVEMENT OF PROTEIN KINASE C IN AGONIST-INDUCED DESENSITIZATION OF NEUROTRANSMITTER RECEPTORS COUPLED TO PHOSPHOLIPASE C. D.-M. Chuang and O. Dillon-Carter. Lab. of Preclinical Pharmacology, National Institute of Mental Health, St. Elizabeths Hospital, Washington, D.C. 20032.

Primary culture of cerebellar granule cells express muscarinic cholinergic, α_1-adrenergic, serotoninergic 5-HT_2 and histaminergic receptors coupled to phosphoinositide (PI) hydrolysis. Exposure of granule cell neurons to a receptor agonist for each of these receptors resulted in time-dependent desensitization of the receptor-mediated PI response. The muscarinic receptor was relatively resistant to desensitization and the desensitization appeared to precede the loss of receptor binding sites. Although phorbol esters effectively inhibited the PI response mediated by each of these receptors, several lines of evidence speak against the involvement of protein kinase C in the agonist-induced desensitization. Thus, incubation of cells with a protein kinase C inhibitor H_7 (25-100 μM) prior to preexposure with carbachol, NE, 5-HT or histamine, did not affect agonist-induced desensitization. Depletion of majority of protein kinase C in granule cells by 24 hr incubation with a phorbol ester, PMA or PDB, also did not alter agonist-induced desensitization. Moreover, agonist-induced desensitization was detected even when cells were prestimulated with agonists at 4°C; at this temperature, agonist-induced PI breakdown was completely arrested.

389.4

PHORBOL ESTER INHIBITS AGONIST-INDUCED 3H-INOSITOL PHOSPHATE FORMATION BY ACTING ON A TARGET OTHER THAN PROTEIN KINASE C. S.V. Bhave*, R.K. Malhotra*, T.D. Wakade*, A.R. Wakade (Spon. R. Gala). Dept. of Pharm. Wayne State Univ., Detroit, MI 48201

It has been suggested that protein kinase C (PKC) is negatively coupled to phosphoinositide hydrolysis because phorbol esters inhibit agonist induce inositol phosphate formation. This hypothesis was investigated by using other agents known to activate PKC via membrane receptors. Formation of ^{3}H-inositol monophosphate (^{3}H-IP) was estimated in embryonic chick sympathetic neurons maintained in culture. Acetylcholine (ACh, 100 uM) caused 10-fold increase in ^{3}H-IP formation. Phorbol 12,13-dibutyrate (PDB) inhibited ACh-induced ^{3}H-IP formation in a dose-dependent manner. However, activation of PKC by serotonin (5-HT, 1 uM), ACh (100 uM) or muscarine (100 uM) had no inhibitory effects on ACh-induced ^{3}H-IP formation. Inhibitory effects of PDB on ACh-induced ^{3}H-IP formation persisted even in presence of H_7 (1 uM) or sphingosine (100 uM) which completely blocked PKC activity. Since other activators of PKC failed to mimic and since inhibitors of PKC failed to block the effects of PDB on phosphoinositide hydrolysis, we suggest that phorbol esters may act on sites other than PKC to modulate neuronal metabolism.

389.5

EFFECTS OF PHORBOL DIESTER ON THE INTERACTION OF ADRENOCEPTORS AND ADENYLYL CYCLASE IN RABBIT UTERINE MYOCYTES: MODULATION BY SEX STEROIDS. J. M. Savola* and J. M. Roberts. Reprod. Endocrinol. Center and C. V. R. I., Univ. California, San Francisco, CA 94143.

Previous studies from our laboratory indicate that the sex steroids, estrogen and progesterone, modulate contractility and other functions regulated by adrenergic β and α_2-adrenoceptors in the rabbit uterus. To study whether this modulation involves an action of the sex steroids on protein kinase C (PKC), we injected ovariectomized rabbits with sex steroids (with estrogen 50 μg/kg i.m., 4 days, or after a priming 4-day-period with estrogen with progesterone 5 mg/kg i.m., 4 days), isolated uterine myocytes, and measured β and α_2-adrenoceptor-mediated effects on adenylyl cyclase activity after 16 h in culture. We measured adenylyl cyclase activity using a [^{3}H]adenine-prelabeling technique, isolating [^{3}H]cAMP by sequential Dowex and alumina column chromatograhy. We found that activation of PKC with phorbol 12-myristate 13-acetate (1 μM, 1 h) in myocytes from rabbits injected with vehicle, estrogen, or progesterone impaired the increase in adenylyl cyclase activity induced by the β-adrenoceptor agonist isoproterenol. Furthermore, this effect was greater in the cells from the rabbits not injected with the sex steroids. In contrast, in the all groups PKC activation had no significant effect on the ability of epinephrine to activate α_2-adrenoceptors and inhibit adenylyl cyclase stimulated by forskolin or prostaglandin E_2. In conclusion, our results indicate that PKC activation impairs the β but not α_2-adrenoceptor-related signal transduction pathway of uterine myocytes, and this effect is modulated by the sex steroids.

389.6

MODULATION OF 5-HYDROXYTRYPTAMINE$_{2A}$ RECEPTOR DENSITY BY GUANINE NUCLEOTIDES. M.A.Harrington and S.J.Peroutka. Department of Neurology, Stanford University School of Medicine, Stanford, CA 94305.

The 5-hydroxytryptamine$_{2A}$ (5-HT_{2A}) is a subtype of the 5-HT receptor which is selectively labeled by ^{125}I-R-(-)2,5-dimethoxy-4-iodophenylisopropylamine (^{125}I-R-(-)DOI), ^{77}Br-R-(-)-4-bromo-2,5-dimethoxyamphetamine (^{77}Br-DOB) and ^{3}H-DOB. Radioligand binding studies were used to study the effect on affinity (K_D) and B_{max} of ^{125}I-DOI binding in rat cortical membranes after pre- or co-incubation with 10^{-4} M ATP, GTP or GTPYS. Co-incubation with either GTP or GTPYS significantly increased the K_D by 57 $\pm$ 10%, while co-incubation with GTP and GTPYS significantly reduced the B_{max} of ^{125}I-DOI binding by 37 $\pm$ 10% and by 51 $\pm$ 10%, respectively. Co-incubation with ATP increased the K_D and reduced the B_{max} of ^{125}I-DOI binding, but the changes were not significant.

The change in B_{max} observed in the presence of GTP and GTPYS is completely reversible as shown by pre-incubation studies. Pre-incubation of the membranes with GTP produced no significant change in either the K_D or the B_{max} of ^{125}I-DOI binding. By contrast, pre-incubation with GTPYS has no effect on the B_{max}, but significantly increased the K_D by 55 $\pm$ 15%. These data differ considerably from the results of analogous experiments performed with the 5-HT_{1A} and 5-HT_{1D} receptors, and may supprt a different mechanism of regulation of 5-HT_{2A} receptor density and affinity through the actions of G proteins.

389.7

CELLULAR ADAPTATION TO OPIATE EXPOSURE INVOLVES AN ADDITIONAL RECEPTOR-BASED PROCESS DISTINCT FROM DESENSITIZATION AND DOWNREGULATION. M v Zastrow*, J D Barchas and C J Evans, Pritzker Laboratory of Behavioral Neurochemistry, Stanford University.

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

389.8

ENDOGENOUS MODULATORS FOR BRAIN L-GLUTAMATE AND GABA RECEPTORS. C.C. Liao,*[2,3] C.J. Lin,*[2,3] Y.H. Lee,*[1] J.Y. Ho,*[1] W.H. Tsai,*[1] and J.-Y. Wu.[1,2,3] [1]Inst. Biomed. Sci., Academia Sinica, Taipei, Taiwan; [2]Neurosci. Program & Dept. Anatomy, Penn St. Univ., Hershey, PA 17033; [3]Dept. Physiol. & Cell Biol., Univ. Kansas, Lawrence, KS 66045

It is known that the activity of L-glutamate receptors (GluR) and GABA receptors (GABAR) is greatly enhanced by repetitive freezing, thawing, and washing of the membrane preparations suggesting the presence of endogenous modulators (EM) that can modify the receptor activities. Indeed, when the membrane extracts were added to the binding mixture, they inhibited the binding of ^{3}H-L-Glu/^{3}H-muscimol to the receptor. The EM for GluR were separated into two components, one as activators and the other one as inhibitors, on Bio-gel P2 column. However, the EM for GABAR were found to be only inhibitory. The EM for GluR and GABAR are distinctly different from L-Glu or GABA itself as judged from the elution profile of standard ^{3}H-L-Glu or ^{3}H-GABA and that of the EM in both Bio-Gel P2 and HPLC C_{18} columns. The activators and inhibitors appear to have a molecular weight of 2,000-10,000 daltons and of 100-2,000 daltons, respectively. The activators are heat and acid sensitive while the inhibitors are quite stable. The EM for GABAR appear to be quite specific for GABA binding site since they have little effect on the diazepam binding site. (Supported by grants from National Science Council, Taiwan and grants NS 20978, NS 20922, and EY 05385 from NIH, U.S.A.)

389.9

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

The effects of cholecystokinin-8 (CCK; 0.3-30 nM) and neurotensin (0.1-30 nM) *in vitro* were investigated on the binding of ^{3}H-N-propylnorapomorphine (^{3}H-NPA, a D-2 agonist *in vitro*) in cryostat sections and in membrane preparations of 8 postmortem normal human basal ganglia. CCK decreased the binding of 250 pM ^{3}H-NPA in the caudate by 50 % at 3 nM. Similar, bot not statistically significant modulations were seen in the putamen and in the nucleus accumbens. In membranes from the caudatus-putamen CCK increased the K_D of ^{3}H-NPA by 30 % at 10-30 nM, without affecting the B_{max}-value. Neurotensin also increased the K_D without affecting the B_{max} of ^{3}H-NPA. The maximal increase of 45 % was obtained at 3 nM of neurotensin. The induction of a reduced affinity of D-2 receptors by CCK and neurotensin are in agreement with results obtained in the rat, and suggest the presence of intramembrane modulation of D-2 receptors by CCK and neurotensin in the living human brain. This receptor-receptor interaction may be of importance for the pathophysiology and treatment of schizophrenia and tardive dyskinesias.

389.10

CHRONIC INTRATHECAL BACLOFEN REDUCES $GABA_B$ BINDING IN RAT SUBSTANTIA GELATINOSA. J.S. Kroin*, R. Singh*, R.D. Penn, and G.D. Bianchi*. Dept. of Neurosurgery, Rush Medical College, Chicago, IL 60612

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

Normal rats were implanted with chronic intrathecal catheters and infused for four weeks with either racemic baclofen (0.5 μg/h) or saline vehicle using osmotic minipumps. The dosage chosen was the highest one that did not produce hindlimb muscle weakness. At the end of the infusion period, lumbar spinal cord sections were prepared for receptor autoradiography (Bowery N.G., et al., Neurosci., 20:365, 1987). In the substantia gelatinosa, the spinal region where $GABA_B$ binding sites have the greatest density, the number of sites was reduced by 36% in the baclofen infused animals as compared to the vehicle controls. The results indicate that there is a down-regulation of $GABA_B$ receptor numbers in the rat spinal cord following chronic high-level exposure to baclofen, and this may be the primary reason for tolerance in patients.

389.11

CHANGES IN MESOLIMBIC DOPAMINE FUNCTION FOLLOWING HIPPOCAMPAL KINDLING. J.G. Csernansky, C. Coronel-Bell*, E. Petrie*, L. Lombrozo*. Laboratory of Clinical Psychopharmacology, Stanford University, Stanford, CA 94305.

We have previously reported that two weeks following electrical kindling of the left hippocampus, a two-fold upregulation of dopamine (DA) D2 receptors in the ipsilateral nucleus accumbens occurs. (Csernansky et al., Brain Res., 449:357, 1988). In the present experiment, we sought to determine whether a concomitant change in presynaptic DA function also occurs.

DA turnover was assessed in rats kindled in the left hippocampus and controls two weeks following the last kindled seizure. In kindled rats compared to controls, a significant 26% decrease in dihydroxyphenylacetic acid (DOPAC) and a 22% decrease in homovanillic acid (HVA) concentrations were observed in the ipsilateral nucleus accumbens. No changes in DA concentrations in the ipsilateral nucleus accumbens, and no changes in either DA, DOPAC, or HVA in the contralateral nucleus accumbens were observed. In the ventral tegmental area, significant bilateral 100% increases in DA concentrations and 50% increases in DOPAC and HVA concentrations were observed in kindled vs. control rats. No changes were observed in the substantia nigra. These data suggest that hippocampal kindling has relatively long-lasting effects on DA synthesis and turnover in the area of mesolimbic cell bodies as well as their terminal fields.

390.1

ANALYSIS OF NEONATAL AND ADULT RAT OLFACTORY BULB GLIAL CELL LINES. M. N. Goodman*, J. Silver, J. W. Jacobberger* (SPON: R. C. Wilcott). Dept. of Genetics, Case Western Reserve Univ., Cleveland, OH 44106.

The rat olfactory bulb is reinnervated throughout life unlike other regions of the central nervous system, where glial differentiation affects loss of plasticity. In vitro, the differentiated phenotype of glial cells can be preserved by cell immortalization via oncogene transduction. We have used this approach to facilitate a study of the roles of glia in the innervation of the olfactory bulb.

Immortalized cell lines have been established from cultures of rat neonatal and adult olfactory bulbs and characterized concurrently with analogous primary cells. Two distinct phenotypes are evident within both neonatal and adult primary cultures: stellate, type I astrocyte-like, and fusiform (Anat. Record 210:385, Dev. Biol. 130:237) cells, each containing glial filaments. Both phenotypes are also present in uncloned immortalized cultures of neonatal and adult bulb.

Neurite outgrowth over monolayers of immortalized uncloned neonatal cultures as well as stellate and fusiform immortal neonatal clones has been compared with outgrowth over immortalized immature (postnatal day 1) and mature (adult) cerebral cortical astrocyte cultures. In assays of 24-hour neurite outgrowth from stage 31 chick retinal neurons, mean neurite length was equivalent for neonatal olfactory cell lines and the immature cortical line. In contrast, the mean length for the mature cortical astrocyte line was reduced by 40% which is close to the 30% reduction seen in primary immature and mature cortical astrocyte cultures (Smith, Silver, Rutishauser, and Miller, submitted). These results suggest that: 1) the neurite promoting properties of glial cells are preserved through immortalization, and 2) the neurite promoting properties of neonatal olfactory and cerebral astrocytes are similar. Assays of neurite outgrowth over adult olfactory glial cell lines are in progress.

390.2

EXPRESSION OF GLIAL FIBRILLARY ACIDIC PROTEIN IN THE RAT MAIN OLFACTORY BULB FOLLOWING SURGICAL AND CHEMICAL LESIONS OF THE OLFACTORY NERVE. M.R. Poston*, M.S. Bailey and M.T. Shipley (SPON: G.C. Blaha). Dept. of Anat. and Cell Biol., Univ. of Cincinnati, Cincinnati. OH 45267.

Severe injury to the mammalian olfactory nerve (ON) results in deafferentation of the main olfactory bulb (MOB), including changes in the glial environment. It is unclear whether axons of regenerating primary olfactory neurons (PONs) encounter a glial environment permissive to growth or must overcome a non-permissive environment. In this study, we observed the expression of glial fibrillary acidic protein (GFAP) by MOB astrocytes at timed intervals following deafferentation of the adult rat MOB. Unilateral deafferentation was performed by 1) surgically severing the ON at the cranial surface of the cribriform plate, and 2) chemically lesioning PONs with zinc sulfate placed in the olfactory epithelium. A polyclonal antibody to GFAP was used to visualize GFAP expression immunohistochemically in the MOB. At 4 days, the amount of GFAP expression in the deafferentated MOB was significantly greater than that in the control MOB. GFAP-positive, hypertrophied astrocytic processes were prevalent in the deafferentated MOB but not in the control MOB. Previous electron microscopic studies have suggested that hypertrophied glial (astrocytic) processes in the MOB are not evident by the tenth day following a lesion of the ON. However, in this study the increased GFAP expression in the deafferentated MOB was still observed at 14 days following both types of lesion. Studies to determine if GFAP expression decreases at longer survival times are in progress. (Supported by NS 23348 and U.S. Army DAMD 17-86-C-6005)

390.3

PLASTICITY OF LHRH-IMMUNOREACTIVE FIBERS IN ACCESSORY OLFACTORY PATHWAY. M. Ichikawa[1] and Y. Oka[2]. [1]Dept. Anat. Embryol., Tokyo Metro. Inst. for Neurosci., Tokyo 183 and [2]Zool. Inst., Fac. of Sci.,Univ. of Tokyo, Tokyo 112.

Recently, we showed a reorganization of neuronal connections in medial amygdaloid nucleus (MAN) following accessory olfactory bulb (AOB) lesion in adult rat. On the other hand, it has been known that the LHRH-immunoreactive (LHRH-IR) fibers are distributed in MAN. In the present study, we examined, using the immunohistochemical technique, if the LHRH-IR fibers in the MAN undergo the plastic changes after AOB removal. Under anesthesia, rat AOB was removed unilaterally and the rats were perfused at 6 hrs, 4 days, or 2 months survival time. The sections including the MAN was processed by the immunohistochemical method. The LHRH-IR fibers were clearly recognized in the MAN. The total length of LHRH-IR fibers in MAN on the side of AOB removal was compared with that on the intact side. Total length of LHRH-IR fibers increased significantly on the side of AOB removal at 4 days and 2 months survival time in each animal. This indicates the possibility that the sprouting of LHRH-IR fibers take place in MAN after the denervation of AOB fibers.

390.4

NEURAL REGULATION OF VASOPRESSIN PROTEIN AND mRNA LEVELS IN SUPRAOPTIC AND PARAVENTRICULAR NUCLEI AFTER MEDIAL FOREBRAIN BUNDLE LESIONS. C. J. Phelps, S. W. Carlson*, and D. L. Hurley*. Dept. Neurobiology & Anatomy, Univ. Rochester Med. Ctr., Rochester, NY 14642.

Vasopressin (VP) - secreting neurons of the hypothalamic supraoptic nucleus (SON) receive a dense noradrenergic (NE) innervation from medullary cells via the medial forebrain bundle (mfb). Electrophysiological studies have shown a stimulatory role for this innervation (Day and Renaud, Brain Res. 303: 233, 1984); mechanical deafferentation of SON leads to decreased peripheral VP correlated significantly with decreased NE levels in SON (Phelps, Carlson, Felten, Anat. Rec. 222: 223A, 1989). In order to investigate the mechanism of this neural regulation, VP expression in SON and paraventricular nucleus (PVN) was investigated by simultaneous immunocytochemical (ICC) and in situ hybridization morphological methods, and by monitoring peripheral secreted VP levels. Young adult (3m.o.) and aged (20m.o.) rats were subjected to SON-deafferenting mfb lesions. Urinary VP levels were monitored before and after surgery. Peripheral VP was decreased in aged, but not in young, rats. VP and VP mRNA were assessed simultaneously at 4, 14, 30 and 60d after lesion using "cryoprotected" fixed frozen thick sections of hypothalamus. SON VP protein (by ICC) was markedly decreased at all intervals in both age groups after lesion; VP mRNA was unaffected in either age group. In young rats, PVN VP protein and mRNA were visibly intensified; "intermediate" (between PVN and SON) VP cell groups, obscure in intact hypothalamus, were visualized using both techniques. A PVN compensatory activity was thus implied to explain "control"-level urinary VP. Since low peripheral VP levels precluded increased VP turnover, the collective results indicate a translational effect of NE deprivation, and a transcriptional effect in PVN compensatory VP elevation. Supported by NIH grant AG06139 (CJP).

390.5

MEDIAL PREOPTIC AREA KINDLING INDUCES SEXUAL BEHAVIOR IN SEXUALLY INACTIVE MALE RATS. A.E. Haller*, M.C. Manero*, R. Paredes*, R. Alvarado* and A. Agmo. Department of Psychology, Universidad Anahuac and Reticular Formation Lab., Instituto Nacional de Neurologia, Mexico, D.F.

In the present experiments, sexually experienced male rats were kindled in the medial preoptic area (MPOA) or the amygdala (AMG) with the purpose to investigate if the widespread modification of brain function produced by kindling induces sexual behavior in noncopulating rats and if kindling facilitates sexual behavior in copulating rats. The animals were stimulated 4 times daily on odd days and sexual behavior monitored on even days. The results showed that the MPOA required higher stimulus intensity to elicite an afterdischarge (AD) than the AMG. No difference was found in the development of kindling between these brain structures.

Kindling had no effect upon sexual behavior in copulating rats. In contrast, MPOA kindling induced copulation in noncopulating male rats. Seven out nine animals displayed sexual behavior. No significant facilitation was observed after AMG kindling in noncopulating male rats. Furthermore, the sexual behavior displayed by the previously noncopulating MPOA kindled rats was similar to the sexual behavior displayed by sexually experienced animals. It is proposed that sexual behavior is facilitated in these rats by local neural changes produced by kindling.

390.6

THE USE OF MICROWAVE IN FIXATION OF BRAIN TISSUE FOR IMMUNOELECTRON MICROSCOPY. J. Anderson, II*, M. Morales and E. Fifkova. Neuroscience Center, Department of Psychology, University of Colorado, Boulder, CO 80309.

It is generally accepted that vascular perfusion fixation with a standard aldehyde mixture is the only adequate method for preparing brain tissue for electron microscopy. However, perfusion is not always possible, e.g., in the case of human material or brain slices. Fixation by immersion is an alternate possibility. However, in the brain it yields preservation which is inferior to that obtained by perfusion. Recently it has been reported that immersion into 6% glutaraldehyde combined with microwave irradiation yields very good results (Jensen and Harris, *Soc Neurosci. Abstr.*, 550, 1988). We have adapted this procedure for use in immunocytochemistry. Blocks of brain tissue were immersed into a mixture of 4% paraformaldehyde and 1-1.5% glutaraldehyde for 20 and 40 sec of uniform heating to 50° C. The tissue was postfixed with uranyl acetate instead of OsO_4 and embedded at low temperature in Lowicryl K4M. The actin filaments were well preserved. In order to use this procedure for immunoelectron microscopy, we had to establish that antigenicity of the tissue did not suffer by the microwave irradiation. Therefore, we used monoclonal antiactin antibodies with a gold probe which were previously tested on perfused material. In comparing the pattern of actin labeling with the two fixation procedures, we could not detect any differences in the distribution of the gold label. Thus, it is possible to use a combined procedure of immersion fixation, microwave irradiation, and low temperature embedding for immunoelectron microscopy.

Supported by NIH Grant AG04804.

390.7

DISTRIBUTION OF MYOSIN IN THE CNS IDENTIFIED WITH MONOCLONAL BRAIN ANTIMYOSIN ANTIBODIES. M. Morales and E. Fifkova. Neuroscience Center, Department of Psychology, University of Colorado, Boulder, CO 80309.

Previously we have described that polyclonal human antiplatelet myosin antibody cross-reacts with brain myosin in rats and mice. This antibody recognizes myosin in dendritic spines, axon terminals, and in dendrites in an area subjacent to the postsynaptic density. With this antibody, we could not establish with certainty whether myosin is also present in other parts of the dendrites and axons. Since the antiplatelet myosin antibody may not have recognized myosin in all compartments of the neuron, we have developed monoclonal antibrain myosin antibodies using the rat brain myosin as an immunogen (in collaboration with the Cancer Center, Health Sciences Center, University of Colorado, Grant P30-CA46934). The clones were screened for a positive reaction on tissue by immunoelectron microscopy and the specificity of the antibodies was tested on immunoblots (Morales and Fifkova, *J. Comp. Neurol.*, 279:666, 1989). With these monoclonal antibrain myosin antibodies, we confirmed the presence of myosin in the dentate fascia, hippocampus, visual and cerebellar cortices, and its distribution in dendritic spines, axon terminals and dendrites where it was associated with actin filaments. In addition, we have established that myosin is also associated with microtubules in dendrites and axons. While myosin, in association with actin filaments in dendritic spines, may play an important role in the mechanism of synaptic plasticity, its association with microtubules in axons and dendrites may indicate a transport-providing function.

Supported by NIH grant AG04804.

390.8

KINDLING-INDUCED INCREASE IN DIMENSIONS OF PERFORATED POSTSYNAPTIC DENSITIES. Y. Geinisman, L. deToledo-Morrell and F. Morrell. Dept. of Cell Biol. & Anat., Northwestern Univ. Med. Sch. and Depts. of Neurol. Sci. and Psychol., Rush Med. Coll., Chicago, IL 60611.

Kindling, which is associated with an exceptionally enduring augmentation of synaptic efficacy, is likely to involve structural alterations of synapses such as increases in their number and size. This study was designed to elucidate whether kindling induces an increase in dimensions of the postsynaptic density (PSD) which delineates the most concentrated area of postsynaptic neurotransmitter receptors and ion channels. Rats kindled via medial perforant path stimulation (1 msec pulses at 60 Hz, twice daily) were sacrificed 4 weeks after reaching a criterion of 5 generalized seizures. Unkindled but stimulated (coulombic control) and unstimulated but implanted rats served as controls. A differential analysis of perforated axospinous synapses with discontinuous PSDs and of nonperforated ones with continuous PSDs was performed in the middle (MML) and inner (IML) molecular layer of the dentate gyrus. For each PSD, all profiles were measured in serial sections to assess the maximal profile length and area (from the total profile length and section thickness estimated with the small-fold technique). The results showed that both the maximal profile length and the area of PSDs were selectively increased following kindling only in perforated synapses of the MML. In marked contrast, nonperforated synapses in the MML were not changed with respect to the measures used. In the IML, however, PSD dimensions were not altered in either of the two synaptic types. Thus, the observed modification is a manifestation of synaptic plasticity induced by kindling rather than a result of generalized seizures. Since perforated synapses are believed to augment the efficiency of transmission, the expansion of their PSDs may represent a structural rearrangement particularly appropriate for the sustained enhancement of synaptic efficacy which characterizes kindling.

Supported by Grants BNS 86-07272 and 87-19107 from NSF.

390.9

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

One of the major problems in identifying biochemical or molecular biological mechanisms involved in the kindled state has been dissociating changes due to acute ictal events from those responsible for the permanent alterations in seizure susceptibility produced by kindling. Seizure control procedures are necessary to make this dissociation. The ideal procedure should elicit behaviorally identical seizures in naive animals in a single brief trial, and should not result in positive transfer to kindling. In this study we have evaluated 3 seizure induction procedures electroconvulsive shock (ECS), low frequency train (LFT) stimulation to the amygdala and low frequency pulse (LFP) stimulation to the frontal cortex, against these criteria.

Amygdala implanted rats (6-10/group) were pretreated with one of the seizure induction procedures and following a 1-2 day rest were kindled using standard protocols. ECS elicited seizures were behaviorally distinct from kindled seizures, and did not result in positive transfer. LFT elicited self-sustained stage 5 seizures after 6.6±2.0m and resulted in robust positive transfer. LFP elicited self-sustained stage 5 seizures after 15.1±2.8s and did not result in positive transfer to amygdala kindling. The relative merits of these procedures will be discussed.

390.10

EVIDENCE FOR A STEPWISE PROGRESSION OF KINDLED SEIZURE DEVELOPMENT. J.L. Burchfiel and C.D. Applegate. Department of Neurology, The Children's Hospital, Boston, MA 02115.

Our investigations with the kindling antagonism model suggest that kindled seizure development is a stepwise process involving two discrete transitions or "gates" from one state of neural organization to another (Burchfiel & Applegate, Neurosci. Biobeh. Rev.,1989). Our data suggest that there is a forebrain gate which controls the transition from stage 1-2 to stage 3 and a brainstem gate which controls the transition from stage 3 to stage 4-5. If these gates control discrete steps in the kindling process, then one would predict that they could be opened independently. We tested this prediction. Rats (N=9) were pretreated by low frequency (2/s) train stimulation applied to the amygdala. This procedure produced stage 5 seizures in all animals with a latency of 3.6±0.3m. Following this, kindling from the entorhinal cortex showed a major acceleration of the transition from stage 3 to stage 4-5 ($\bar{x}$ trials=3.4±0.5 v. 9.2±1.1 in controls); whereas, there was no alteration of the transition from stage 1-2 to stage 3 ($\bar{x}$ trials=16.1 ±2.7 v. 12.6±1.7 in controls). These findings confirm our prediction of independence of the two transitions, support the hypothesis that kindling is a stepwise process and indicate that the amygdala is downstream from the forebrain gate.

390.11

ATTENUATION OF GENETIC RESPONSE TO SEIZURES FOLLOWING KINDLING. C.D. Applegate, L.R. Dawes, G. Samoriski, R.L. Neve. Harvard Medical School, Boston, MA 02115.

Research from our laboratory has indicated that baseline transcriptional activity for a variety of mRNAs is unchanged in cerebral cortex (CX) and hippocampus (HPC) following kindling. In this study we examined regional mRNA levels for fos and the alpha subunit of Ca^{+2}/calmodulin dependent protein kinase II (CaMII) in kindled and naive animals at 0.5, 1.0, 6.0 and 24 h following seizures (N=2-4/group/time). Rats were kindled from the amygdala using standard protocols until 6 consecutive stage 5 seizures were elicited. Stage 5 seizures were elicited in naive animals by 2/s, high frequency train stimulation of the amygdala. Fos mRNA levels in CX and HPC were significantly elevated at 0.5 and 1.0 h following seizures in both kindled and naive rats; however, the magnitude of this increase was significantly greater in naive (OD = 4.0±1.1) vs. kindled (OD = 1.00 ± 0.03) rats in the HPC at both time points. CaMII levels were significantly increased in naive but not kindled HPC 0.5 h post-seizure, but were not changed in either group in CX. These data may suggest that kindling attenuates the responsiveness of fos and CaMII mRNAs to seizures. However, a contribution of the seizure induction procedure in naive animals cannot be ruled out. Experiments are currently being conducted to resolve this issue.

390.12

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

Unilateral, electrolytic lesions of the hippocampal dentate gyrus hilus produce recurrent limbic seizures which begin 2 hrs postlesion and recur for 8-10 hrs. Electron microscopic analysis has demonstrated that during this period of seizure activity there is a dramatic increase in the number of spines on the somata of the dentate gyrus granule cells. At 5 and 11 hrs postlesion there were 1.80 ± 0.10 (mean ± S.E.M.) and 1.59 ± 0.32 spines/cellular profile, respectively verses 0.19 ± 0.07 spines/cellular profile in controls. In the present study the persistence of these spines was examined in rats sacrificed 4 and 14 days following one hilus lesion-induced seizure episode. There are 0.83 ± 0.13 and 0.89 ± 0.07 spines/cellular profile at 4 and 14 days, respectively. These data were taken from analyses of 91 cellular profiles in 4 animals at 5 hrs; 88 cellular profiles in 4 animals at 11 hrs; 36 cellular profiles in each of 3 animals at 4 days and 14 days; and 118 cellular profiles in 6 control animals. Thus, approximately 50% of the somatic spines present during the period of seizure activity appear to be lost by 4 days post-seizure but the remaining 50% persist at least 14 days and possibly longer. While approximately 40% of the somatic spines found during the period of seizure activity are quite large and complex, sometimes completely engulfing presynaptic elements, those that remain at 4 and 14 days are all much smaller and 'nub' shaped. This latter population of spines are of the same morphological type found in control animals but are 4 times as numerous as seen in control rats.

These studies suggest that the increased neural activity experienced during recurrent seizures has long term structural effects in adult CNS neurons.

This work was supported by NINCDS NS26748.

390.13

EFFECTS OF POSTOPERATIVE ENRICHMENT ON BEHAVIORAL RECOVERY ARE BOTH LESION- AND TASK-DEPENDENT. C.Kelche* and B.E.Will (SPON : N.Ropert). D.N.B.C., Centre de Neurochimie du CNRS, 12 rue Goethe, 67000 Strasbourg, France.

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

In the present study, we examined the effects of postoperative environment on the behavior of rats with either entorhinal cortex or dorsal hippocampus lesions on different behavioral tests: spontaneous alternation in a T-maze, learning of an eight-arm radial (EAR) maze and of an Hebb-Williams (HW) maze. Both entorhinal cortex and dorsal hippocampus lesions impaired performance in each of these tasks. Enrichment of the postoperative environment failed to facilitate behavioral recovery of any of the entorhinal cortex lesion-induced effects. Similarly, postoperative enrichment failed to attenuate ($F < 1.0$) the deleterious effects of dorsal hippocampus lesions on spontaneous alternation. However, in the same rats, the "enriched" housing attenuated the deficits observed in the EAR maze just at the $P < 0.05$ level ($F\ 1/21 = 4.4$) and in the HW maze at the $P < 0.01$ level ($F\ 1/21 = 24.47$).

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

390.14

EFFECTS OF TASK DIFFICULTY ON RECOVERY OF SPATIAL PERFORMANCE AFTER UNILATERAL ENTORHINAL CORTEX (EC) LESIONS IN RATS. J.J. Ramirez*, B. Fass-Holmes, K. MacDonald*, J. McClure*, and C. Tuite*. Psych. Dept., Davidson College, Davidson, NC 28036; Biol. Dept., UCSD, La Jolla, CA 92093.

Unilateral EC lesions impair performance on a learned alternation task from which the rats recover 8-10 days postlesion. Since the time course of this recovery parallels the time course of sprouting, these two phenomena are thought to be related (Loesche and Steward, 1977). The objective of the present investigation was to determine whether there are limits within which sprouting may effectively contribute to recovery. We examined the effects of unilateral EC lesions on a spatial alternation task (Y maze) with intertrial intervals of differing lengths (0, 40, 70, 100 sec). Whereas the performance of the 0 sec group was spared, the other groups showed a deficit from which they all required 10-12 days to recover. This finding indicates that the effects of EC lesions on spatial alternation depend upon task difficulty as determined by the length of the intertrial interval. Since the time course of recovery was comparable for the 40, 70, and 100 sec groups, such recovery evidently is independent of task difficulty. The known parallel between the time course of recovery and sprouting after EC lesions therefore implies that the recovery depends upon the sprouting instead.

Supported by NIH grant NS24948 (J.J.R.)

390.15

INJECTION OF TETRODOTOXIN INTO THE ENTORHINAL CORTEX DEPRESSES CELL FIRING IN THE DENTATE GYRUS. R.Tomasulo*, B.Burger*, O.Steward (SPON: P.Trimmer) Dept. of Neuroscience, Univ. of VA, Charlottesville, VA 22908

Ablation of the entorhinal cortex (EC) of the rat induces a complex sequence of dendritic reorganization and axonal sprouting in the denervated dentate gyrus (DG). The signal that triggers these events is unknown. One candidate, however, is the reduction of granule cell firing which follows the EC lesion (Reeves and Steward. Exp. Neurol. 102:37-49, 1988). As a first step in comparing the effects of denervation with the effect of merely silencing the EC input, we recorded granule cell firing before and after injecting tetrodotoxin (TTX) into the EC.

Under urethane anesthesia, we isolated putative granule cells with a tungsten electrode. After defining baseline activity, we injected 0.2 to 0.4 ul of $2x10^{-5}$ M TTX in 0.9% NaCl into each of 14 stereotaxic sites along two parallel tracks in the EC. Cell firing and evoked responses were monitored for 8 hours. We found that TTX injection reduced granule cell activity to the same extent as did EC ablation. There was no recovery of evoked responses or cell firing in 8 hours. Saline injections did not alter granule cell activity.

We conclude that the previously described rate changes reflect, in the short term at least, lost presynaptic drive rather than an indirect metabolic effect of denervation or an effect dependent upon early degenerative events in presynaptic terminals. Biochemical and morphological studies will determine if silencing the EC reproduces the effects of denervation. Supported by NSF BNS-8818766 (OS). RT received NIH postdoctoral training grant 5T32NS07199.

390.16

Low-Frequency Depression Modulates Long-Term Potentiation Of The Perforant Path In Disinhibited In Vitro Dentate Gyrus. P. C. Rinaldi and E. M. Hookano*. Neurophysiology Lab, Div. of Neurological Surgery, College of Medicine, Univ. of California at Irvine, Irvine, CA 92717.

Low-frequency depression (LFD) was studied in the disinhibited in vitro dentate gyrus (rat) to determine its role in modulation of long-term potentiation (LTP) and thus its importance in computational and theoretical considerations of learning and memory. An electrode placed in the trajectory of the perforant path inputs to the granule cells delivered high-frequency stimulation (400 Hz) to induce LTP and low-frequency stimulation (0.5 Hz) to produce LFD. The dendritic field potential was recorded to assess synaptic activity. Following the induction of stable LTP in 11 slices ($\bar{x}$ increase 62%), low-frequency stimulation was delivered to the same inputs for 3 to 10 minutes. During the course of this stimulation synaptic depression averaged 29%. The post-LTP/LFD level indicated that LTP was modified in 8 of 11 slices. The decrease from LTP levels or reversal of LTP averaged 31% and was stable for up to 47 minutes. Compared to control experiments in which 10 minutes of low-frequency stimulation was studied in non-potentiated slices, response amplitude level was significantly reduced when LFD followed LTP. LFD appears to be capable of modifying or reversing LTP in the dentate. It may play a role in modulation of information in neural networks, particularly in extinction or forgetting. (Supported by NIH NS22980-01A1 to PCR.)

390.17

NEONATAL THYROID HORMONE TREATMENT ATTENUATES THE INDUCTION OF LONG-TERM POTENTIATION IN RAT HIPPOCAMPUS. C. Pavlides*, A.I. Westlind-Danielsson* & B.S. McEwen (SPON: J. Winson). The Rockefeller University, NY, 10021.

Thyroid hormone dysfunction at an early stage of development produces marked neurochemical, and morphological changes in the hippocampus. In order to better understand the functional significance of these effects, we examined LTP induction in the dentate gyrus (DG) of thyroid hormone treated rats (N=3) and control rats (N=3).

Thyroid hormone [3, 5, 3'-triiodo-L-thyronine (T3)] was administered to male rats on postnatal days 1, 2 and 4 (0.5 ug/g of body weight, s.c.) at approximately 2 months of age, the two groups of animals were tested for the induction of LTP by stimulating the perforant path (PP) and recording EPSP's in the DG granule cell region.

In both the control and T3 groups, tetanic stimulation of the PP produced a slight increase in the slope of the EPSP (5.93% +/- 4.02 SEM & 7.27% +/- 3.43 SEM, respectively). However, while the population spike of the control group increased significantly (46.5% +/- 3.55 SEM) following tetanization, the population spike of the T3 treated rats decreased significantly (-44.7% +/- 23.0 SEM). The present results suggest that brief T3 treatment at a critical stage of development may have drastic long-term changes in hippocampal physiology and function.

Supported in part by NSF grant BNS 8706053 to J. Winson & grant MH41256 to BSM.

390.18

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

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

390.19

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

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

Suported by NIH grant NS 11892.

LIMBIC SYSTEM I

391.1

GONADAL STERIODS REGULATE DENDRITIC SPINE DENSITY IN HIPPOCAMPAL PYRAMIDAL CELLS IN ADULTHOOD. C.S. Woolley*, E. Gould*, M. Frankfurt and B.S. McEwen (SPON: W. Yates). Lab. of Neuroendocrinology, The Rockefeller Univ., New York, NY 10021.

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

391.2

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

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

Supported by NIH grants NS 15317 and NS 07097.

391.3

THE DEVELOPMENT OF THE SYNAPTIC PAIRED-PULSE PROFILE IN AREA CA1 OF THE RAT HIPPOCAMPAL SLICE PREPARATION. II. STRATUM MOLECULARE. P.G. DiScenna & T.J. Teyler Neurobiology Dept., Northeastern Ohio Universities College of Medicine, Rootstown, OH 44272.

We study the development of the trisynaptic circuit by examining the development of excitatory and inhibitory synaptic transmission in the dentate gyrus and hippocampus proper. While studying the development of paired-pulse facilitation in str radiatum of CA1, we noted that afferents in the distal apical dendritic field showed an unusual paired-pulse profile (PPP;10-5000ms) during development. We observed a triphasic pattern with paired-pulse depression at short interpulse intervals (IPI), facilitation at intermediate IPIs and a depression at long-latency IPIs. This pattern mirrors the synaptic PPP produced by the med. perforant path input to DG rather than the profile in adult CA1. We investigated this effect more closely. The rat hippocampal slice prep and a homosynaptic paired-pulse paradigm (10-5000ms IPI) were used to examine the development of the synaptic PPP in stratum moleculare of area CA1. Population EPSPs were evoked by str. moleculare stimulation at 40-60% max. Our initial results are based on two animals at each age. The paired-pulse effects ((Test/Cond)X100) developed as follows:

	20ms	80ms	200ms	2000ms	5000ms
PN4	64%	77%	78%	64%	83%
PN6	80	93	100	69	93
PN10	128	145	103	83	88
PN15	148	147	116	90	91

391.4

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

We have developed a tectal slice preparation to examine the pharmacology of retinotectal transmission in *Rana pipiens* tadpoles. We use 500µm thick slices of the diencephalon and tectum. By electrically stimulating the optic tract, which runs through the diencephalon, we can record tectal evoked potentials (TEP) similar to those seen in the intact animal. Such TEP's consist primarily of a fast biphasic component, followed by slower positive and negative components. We have identified the fast biphasic component as presynaptic on several criterea: 1) It is relatively insensitive to zero Ca^{2+}-high Mg^{2+} bathing saline, and also to high concentrations of the calcium channel blockers Co^{2+} or Cd^{2+}, 2) It is not abolished by high-frequency stimulation and recovers immediately after a tetanus, 3) it does not exhibit paired pulse facilitation, and, 4) it is largest in superficial tectum. By using similar methods, we conclude that the later components of the TEP are postsynaptic.

Furthermore, low concentrations of NMDA reversibly block the postsynaptic components, while low concentrations of APV reversibly enhance them. Concurrent with the postsynaptic block due to NMDA, there is a marked increase in spontaneous activity in the tectum, and a substantial increase in the evoked presynaptic components. Since the increase in the presynaptic component is not blocked when transmission is blocked by Cd^{2+}, it is specific to presynaptic elements. We hypothesize that NMDA receptors are highly concentrated on inhibitory cells, which, when driven by exogenous NMDA, inhibit the postsynaptic TEP. Preliminary evidence further suggests that GABA may be involved in the actions of NMDA on the TEP.

Supported by NIH grant EY06039.

391.5

EARLY HANDLING / ISOLATION DECREASES ADULT RATS' USE OF PLACE STRATEGIES. J. Willner* and L. Nadel. Dept. of Psychology, Univ. of Arizona, Tucson, AZ 85721.

Recent evidence suggests that environmental events occurring early in postnatal life can have long-lasting consequences for hippocampal function. In the present experiment, pups from litters of Long-Evans rats were handled for 5 min/day on PN 3-7, 9 and 11. They also received an isolation treatment on PN 8, 10 and 12, during which they were separated from the dam and maintained in a heated, lit environment for 8 hr . The pups were then weaned at PN21, and maintained on ad lib food and water in large metal hanging cages with same-sex littermates.

At 6 mo of age, experimental and control rats were trained on an appetitive position discrimination in a T-maze. Following habituation to the maze, the rats were reinforced for choosing the initially non-preferred arm of the maze and trained to a criterion of 8 correct choices in 10 consecutive trials. The rats then received a nonreinforced probe trial with the start arm of the maze rotated 180° away from it's original position to determine whether they were using a "response" strategy or a "place" strategy to solve the discrimination problem.

Rats receiving the handling / isolation treatment did not differ from controls in acquisition of the position discrimination, but were significantly less likely than controls to have used a place strategy to solve the task (36.8% vs. 83.3%, respectively). These data are consistent with previous work (Wilson et al., Beh. Br. Res., 21:223, 1986) showing that young rats display a more "immature" pattern of LTP after early handling / isolation, and suggest that this treatment has long-term consequences for hippocampal function.

391.6

DEVELOPMENT OF SPATIAL LEARNING (OBJECT LOCALIZATION) ABILITY IN INFANT RATS. K. L. Altemus* and C. R. Almli, Depts. Anat. & Neurobiol., Psychol. Pgms. Neural Sci., Occup.Therapy. Washington Univ. Sch. Med., St. Louis, MO 63110.

Spatial learning abilities are thought to be related to the functional status of the hippocampus. The present investigation studied the development of utilization of spatial cues to mediate object localization for albino rats beginning at 15 days of age. Independent groups of rats were tested in a Morris water maze under proximal (visible goal in fixed location), distal (non-visible goal in fixed location), and random (non-visible goal in random location) conditions. Animals were tested on 3 consecutive days with independent groups beginning on postnatal days 15,16,17,18,and 19. Latencies to enter the escape quadrant and latencies to escape were recorded for each session of 12 1-min acquisition trials. Each daily session concluded with a 1 min probe trial, during which escape was impossible. Latencies to enter the quadrant previously containing the platform, and the total time within this quadrant were recorded. Results indicate that spatial learning abilities based on visual cues are present early in development, that performance is influenced by previous experience, and that spatial learning is manifest prior to hippocampal maturation. (Conducted under NIH Guide for Care and Use of Laboratory Animals)

391.7

CONFOCAL SCANNING LASER MICROSCOPY OF INTRACELLULARLY INJECTED AMYGDALA NEURONS. E. W. Kairiss, P. E. Chapman*, C. L. Keenan, Z. Xiang* and T. H. Brown, Dept. of Psychology, Yale University, New Haven, CT 06520.

The amygdala has been implicated in a variety of important innate and learned behaviors. We have been developing the amygdala slice preparation (Keenan et al, *Brain Res. Bull.*, 1988) because virtually nothing is known about the cellular neurophysiology and neuropharmacology of this interesting region of the brain. Current- and voltage-clamp recordings have revealed that some amygdaloid synapses display long-term potentiation (Chapman and Brown, *Soc. Neuro Abst.*, 1988), which could be relevant to learning (Brown et al, *Science*, 1988). Here we report the successful use of confocal scanning laser microscopy to create 3D images of neurophysiologically-characterized amygdala neurons.

Horizontal amygdala slices were prepared as described previously (Chapman and Brown, 1988). Dye injections and recordings were done using microelectrodes containing 5(6)-carboxyfluorescein. Active and passive membrane properties were assessed from the voltage response to current steps. Synaptic properties were assessed by stimulating the external capsule. Following the neurophysiology, slices were cleared in glycerol with 5% n-propyl gallate added to reduce bleaching. Serial optical sections, taken at 1 to 5 μm intervals, were obtained using a confocal scanning laser microscope.

Reconstructions of the optical sections were used to produce a stereo pair or a red/blue stereo anaglyph. Four optically reconstructed neurons, which could be classified as pyramidal-type, had input resistances between 22 and 70 MΩ and resting potentials between -72 and -79 mV. They responded to depolarizing current steps with trains of spikes, burst discharges, or single spikes. The ability to obtain structure-function relationships in this heterogeneous cell population should prove useful in understanding the circuitry and ultimately the mnemonic functions of the amygdala. *(Supported by the Air Force Office of Scientific Research and the Office of Naval Research).*

391.8

CONFOCAL SCANNING LASER MICROSCOPY OF HIPPOCAMPAL NEURONS FROM CULTURED AND ACUTE BRAIN SLICES. C. L. Keenan, E. W. Kairiss, A. C. Greenwood*, A. C. Nobre, L. Rihm*, T. H. Brown, Dept. of Psychology, Yale University, New Haven, CT 06520.

We have been interested in optical methods that optimize spatial and temporal resolution of cellular and subcellular structure in acute and cultured brain slices (Keenan et al, *Brain Res. Bull.*, 1988). Here we report the successful imaging of hippocampal neurons with confocal scanning laser microscopy (see also Kairiss et al, *Soc. Neurosci. Abstr.*, 1989).

Acute hippocampal slices were prepared in the conventional way using a vibratome. Neonatal hippocampal slices were cultured as described previously (Ganong et al, *Soc. Neurosci. Abstr.*, 1988) and maintained in a roller drum for 3 to 6 weeks prior to their use. Intracellular recordings and dye injections were done with microelectrodes containing either lucifer yellow or carboxyfluorescein. Serial optical sections (3.0 - 15.0 μm intervals using a 10X or 20X objective and 0.1 - 1.0 μm intervals using a 40X or 60X objecive) of the fluorescently-labeled neurons were obtained with a confocal scanning laser microscope. Image reconstructions of the optical sections were then displayed as a stereo pair or as a red/blue stereo anaglyph.

These 3D images were particularly effective for visualizing the spatial relationships among dendritic processes and the interdigitation of processes of injected pairs of neurons. We were particularly interested in visualizing clearly the thorny excrescences, which are dendritic spines associated with the mossy-fiber synaptic input. The excrescences were seen to have extremely complex geometries, sometimes consisting of rosettes of small spines protruding from a central shaft.

We conclude that confocal scanning laser microscopy holds great promise for studies of hippocampal neurons and possibly even for real-time analysis of neuronal structure-function dynamics. This method may enable us to determine whether structural changes occur during activity-dependent synaptic modifications, such as long-term potentiation (LTP) (Brown et al, *Science*, 1988). LTP is a candidate synaptic substrate for certain forms of learning. *(Supported by the Air Force Office of Scientific Research and the Office of Naval Research).*

OPIATES, ENDORPHINS AND ENKAPHALINS: ANATOMY AND CHEMISTRY II

392.1

SIGMA RECEPTOR RECOVERY FOLLOWING MODIFICATION BY EEDQ: EVIDENCE FOR DIFFERENT AGONIST AND ANTAGONIST RECOGNITION SITES OR RECEPTOR SUBTYPES G. Battaglia, Department of Pharmacology, Loyola University, Maywood, Il 60153

Sigma receptor recovery following irreversible inactivation by N-ethoxy-carbonyl-2-ethoxy-1,2-dihydroquinoline (EEDQ) was studied. Male SD rats were treated with either vehicle or EEDQ (10 or 20mg/kg, i.p.) and sacrificed at 6 hours or 4 days post-treatment. Other groups of rats were pretreated with 3mg/kg of either the agonist DTG (1,3-di-o-tolyl-guanidine) or the antagonist haloperidol (HAL) prior to 10mg/kg EEDQ to assess recognition site specificity. Sigma receptors in cerebellum were measured using the agonist ^{3}H-DTG (3nM) and two concentrations of the antagonist ^{3}H-HAL (1.2 and 11 nM) to detect changes in receptor affinity and B_{max}. Marked decreases in ^{3}H-DTG binding were observed following both 10mg/kg (48% of control) and 20mg/kg (38% of control) doses. A 39% recovery of ^{3}H-DTG binding was observed by day 4. In contrast, ^{3}H-HAL-labeled sigma receptors were not reduced by either dose of EEDQ. In addition, HAL pretreatment failed to protect against the EEDQ-induced decrease in ^{3}H-DTG binding while DTG pretreatment resulted in a partial protection. The differential effects of EEDQ on ^{3}H-DTG versus ^{3}H-HAL-labeled sigma sites indicate that agonist and antagonist recognition sites differ. These data also suggest that ^{3}H-DTG may label EEDQ-sensitive and EEDQ-insensitive subtypes of sigma receptors.

392.2

PRESYNAPTIC INHIBITION BY OPIOIDS OF GLUTAMATE-MEDIATED SYNAPTIC POTENTIALS IN RAT STRIATAL NEURONS. Z.G.Jiang* and R.A.North, Vollum Institute, Oregon Health Sciences University, Portland, OR 97201.

Intracellular recordings were made from striatal neurons in 30° obliquely cut slices. Fifteen of 25 tested were identified as projection neurons by antidromic activation by a focal electrical stimulus to the globus pallidus. Focal stimulation of cortical projection fibers evoked a excitatory postsynaptic potential (epsp). In 96 of 112 cells this was unaffected by bicuculline (30 μM) but completely blocked by CNQX (10 μM) and APV (30 μM); it was also blocked by low calcium/high magnesium solutions, and by tetrodotoxin (TTX, 1 μM). [Met^{5}]enkephalin (3, 10, 30 & 100 μM) reduced the epsp amplitude by (%) 10.1 ± 6.7 (mean ± SD, n = 9), 24.3 ± 7.9 (9), 34.8 ± 11.1 (43) and 41.6 ± 8.6 (8), with little or no effect on membrane potential or input resistance. Depolarizations evoked by direct applications of glutamate (pressure pulse) were unaffected, whether (n = 3) or not (n = 5) TTX was present. The inhibition by [Met^{5}] enkephalin was completely antagonized by naloxone (1 μM) and was mimicked by both Tyr-D-Ala-Gly-MePhe-Gly-ol (DAGO, 1 μM; inhibition was 23.1 ± 8.4 % (15) and Tyr-D-Pen-Gly-Phe-D-Pen (DPDPE, 1 μM; inhibition was 23.3 ± 13.1 % (17)), though not by U50488H. It is concluded that some fibres providing excitatory synaptic input to striatal neurones express either μ or δ receptors, activation of which inhibits glutamate release.

392.3

ENKEPHALIN, ENDORPHIN AND DYNORPHIN IMMUNOREACTIVE CELLS AND FIBERS IN THE PITUITARY OF THE AXOLOTL. (Ambystoma mexicanum) M. Leon Olea*, M. Sánchez-Alvarez*, A.L. Piña*, M.Briones* and A. Bayón. División de Inv. en Neurociencias Instituto Mexicano de Psiquiatría. Instituto de Investigaciones Biomédicas, UNAM, AP-70228, 04510 México, D.F.

Recently Assai et al.(Neuropeptides, in press) showed that the enkephalin (ENK) content in the pituitary of the axolotl is several fold higher than that found in birds and mammals. In order to elucidate the cellular localization of this ENK and its relations to other opioid peptides in the pituitary, we carried out immunocytochemical studies through the indirect immunofluorescence method using antibodies directed against leu-ENK, met-ENK, dynorphin(1-10) (DYN) and β-endorphin-βLPH (END). Leu-ENK-immunoreactivity (ir) was found in neurohypophyseal fibers and in numerous cells scattered throughout the anterior lobe; here, END-ir cells constitute a separate population concentrated in the caudal pole (END is also present in intermediate cells). DYN-ir was intense in fibers of the neural lobe but undetectable in the anterior one. Met-ENK-ir could be detected in neurohypophyseal fibers but only a few cells were lightly fluorescent in the anterior lobe. From these data and since Pro-ENK-A genes in amphibians may not codify leu-ENK sequences (Martens and Herbert, Nature 310:251,1984), we suggest that putative Pro-ENK-B containing cells could generate leu-ENK, but not DYN, as a hormone in the hypophysis of the axolotl.

392.4

BIOCHEMICAL CHARACTERIZATION AND BRAIN DISTRIBUTION OF ^{3}H-NALOXONE BINDING SITES IN A URODELE AMPHIBIAN. P. Deviche and F.L. Moore. (SPON: S. EMERY). Inst. Arctic Biology, Univ. Alaska Fairbanks, Fairbanks, AK 99775 and Dept Zool. Oregon St. Univ., Corvallis, OR 97331.

Suspensions of partially purified membranes prepared from newt (Taricha granulosa) brains were incubated with ^{3}H-naloxone and brema-zocine HCl (competitor). ^{3}H-naloxone binding was found to be specific and saturable (Kd: 1 ± 0.3 nM; Bmax: 155 ± 39 fmol/mg protein), time- and temperature-dependent, fully reversible, heat- and trypsin- sensitive, dose-dependently and selectively increased by Na^+ in the incubation medium, and displaced by ligands that are selective for μ, δ, or κ opiate receptors. Using in vitro autoradiography, we found that ^{3}H-naloxone specifically and discretely binds to the urodele brain. The highest specific binding was observed in the medial pallium, the medial septum, the amygdala (pars lateralis), and various diencephalic nuclei. No specific binding was detected in the anterior pituitary. The biochemical characteristics and the neuroanatomical distribution of opiate binding sites of newts and of other vertebrates, therefore, present many similarities.

392.5

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

Opioids in dog heart extracts are characterized by opiate radioreceptor assay (RRA) using dog brain membranes, pretreated with Na^+ and GMP. ^{3}H-diprenorphine was used as a non-specific ligand to permit screening for a spectrum of opioids. Scatchard analysis provided computer fits equally consistent with 2 or 3 site models. K_{D1} (0.4 nM) and B_{MAX1} (66 fmol/mg protein) values agree with reports by others for rat brain. IC50s determined graphically [logit (ln %B÷[100-%B]) vs concentration] were 1.8 nM diprenorphine, 8.1 nM etorphine, 8.4 nM naltrexone, 33 nM DYN(1-13), 41 nM DADLE, 50 nM (-)-naloxone, 104 nM DYN(1-8), 164 nM met-enk, 200 nM DYN(1-9), 810 nM DAGO, 2640 nM U50488H, and > 100 μM (+)-naloxone when 1.5 nM ^{3}H-diprenorphine was used. Tissue unknowns were expressed as nM equivalents of etorphine standards. Canine heart was extracted (1 N acetic acid, 0.2% 2-mercaptoethanol, 0.1 N HCl), homogenized (polytron) and centrifuged. Supernatants were further purified by C-18 cartridge (SepPak, Waters). After evaporation of solvents, reconstituted samples were tested in the RRA. Methods to optimize peptide recovery during tissue collection are under evaluation. Preliminary results indicate opioid activity throughout the myocardium with some preferential concentration in the intraventricular septum. Supported by American Heart Association-Texas Affiliate grant #G-165.

392.6

EFFECT OF CATIONS ON GTP REGULATION OF [125I]β-ENDORPHIN BINDING: DIFFERENCES BETWEEN μ AND δ OPIOID RECEPTORS D.E. Selley and J.M. Bidlack. Department of Pharmacology, University of Rochester, Rochester, NY 14642.

The binding of agonists to μ and δ opioid receptors is inhibited by μM concentrations of GTP. However, we have shown that the binding of [^{125}I]β-endorphin ([^{125}I]β-EP) to rat brain membranes was not inhibited by GTP unless monovalent cations were present. These cations promoted GTP regulation of [^{125}I]β-EP binding with their order of potency being: $Na^+ > Li^+ > K^+$. [^{125}I]β-EP binding to δ opioid receptors in NG108-15 cell membranes was inhibited by GTP in the presence of Na^+, but not Li^+ or K^+. These studies have been extended to membranes prepared from the SK-N-SH cell line, which contain predominantly μ opioid receptors. GTP did not inhibit [^{125}I]β-EP binding to SK-N-SH membranes in the absence of cations, but did inhibit [^{125}I]β-EP binding in the presence of NaCl, LiCl, or KCl, with the order of potency being $Na^+ > Li^+ > K^+$. These cations were about equipotent in inhibiting [^{125}I]β-EP binding by themselves. These results are similar to those obtained in rat brain membranes, but differ from those obtained in NG108-15 membranes. [^{125}I]β-EP binding was not inhibited by GTP in the presence of the divalent cations, Mg^{2+}, Ca^{2+}, or Mn^{2+}, though these cations were about 20 times more potent than the monovalent cations in inhibiting [^{125}I]β-EP binding by themselves. Thus, both μ and δ receptors require monovalent cations for GTP regulation of [^{125}I]β-EP binding, but the specificity of this requirement differs between the two types of opioid receptor.

392.7

EFFECTS OF OPIOID RECEPTOR BLOCKADE ON HIPPOCAMPUS DEVELOPMENT IN ADOLESCENT RATS. J. Reyes*, D. Fish* and M.C. Diamond (SPON: N. Peterson). Department of Physiology-Anatomy, Univ. of California, Berkeley, Ca. 94720

Studies have indicated that regions of the CNS are increased in size and cellular content when opioid receptors are blocked by Naltrexone. Our study examines the relationship between opioid receptor blockade, size (thickness) and laterality in the hippocampus of 41-day-old rats. Male and female Long-Evans rats were injected subcutaneously with either saline, 1, 10, or 50mg/kg of Naltrexone, an opioid receptor antagonist, for the first 41 days of postnatal life. On day 41 rats were sacrificed and morphometric analysis of the hippocampus were made on transverse, frozen 40mu sections stained with a modified Thionine stain. Results indicated increases in male hippocampal thickness at each dosage ($p<0.05$) when compared to control (saline) rats. Furthermore, significant laterality differences were seen in the 1 mg/kg dosage group, with the left hippocampus being larger than right ($p<0.05$). These results are opposite to those seen in male rats treated for the first 21 days postnatally. Female rats showed no significant differences between dosage groups in hippocampal size or laterality. However, as the dosage of Naltrexone increased, the left hippocampus progressed from smaller than controls at the 1 and 10 mg/kg dosages to larger than controls at 50 mg/kg. These results are similar to what is seen in females exposed for 21 days postnatally to Naltrexone.

392.8

STIMULATION-INDUCED RELEASE OF ENDOGENOUS OPIOID PEPTIDES CAUSES DISPLACEMENT OF MU AND KAPPA LIGAND BINDING IN THE HIPPOCAMPUS. J.J. Wagner*, J.F. Neumaier, R.M. Caudle and C. Chavkin, Dept. of Pharmacology, Univ. of Wash, Seattle, WA 98195.

Previous studies have demonstrated that the hippocampus has two major pathways containing endogenous opioid peptides: the perforant path from the entorhinal cortex and the mossy fibers from the dentate gyrus. To determine the physiological conditions required to release opioids from these pathways and the anatomical sites within the tissue that could bind the released opioids, we used a radioligand displacement paradigm in which either 10 nM [^{3}H]-DAGO or 3 nM [^{3}H]-U69,593 was added to incubation wells containing hippocampal slices (500 μm) in oxygenated buffer at 34°C. We found that the specific binding of [^{3}H]-DAGO to mu receptors in rat hippocampal slices was reduced by depolarization caused by 3 μM veratridine or by focal electrical stimulation. [^{3}H]-DAGO displacement was calcium-dependent, blocked by 1 μM tetrodotoxin, and dependant on the presence of peptidase inhibitors. Electrical stimulation of opioid containing pathways caused a reduction in radioligand binding in discrete areas of the hippocampus as shown by quantitative receptor autoradiography. Similarly, [^{3}H]-U69,593 binding to kappa-1 sites in guinea pig hippocampal slices was reduced by both veratridine and electrical stimulation. These results indicate that opioid peptides released under physiological conditions bind to mu and kappa receptors in the rodent hippocampus. The pattern of displacement suggests that opioids can diffuse to distant sites of action under these conditions. Supported by DA-01423 and NS-023483.

392.9

KAPPA OPIOID RECEPTOR-MEDIATED PHOSPHOINOSITIDE TURNOVER IN RAT BRAIN. S. Periyasamy* and W. Hoss (SPON: H. Rosenberg), Department of Medicinal and Biological Chemistry, University of Toledo, Toledo, OH 43606

Both biochemical and pharmacological evidence support the existence of at least three subtypes of opioid receptors μ, δ, and κ in the CNS. Opioid receptors, including κ-receptors, are associated with the inhibition of adenylyl cyclase, stimulation of low-K_m GTP hydrolysis and more recently with the regulation of K^+ and Ca^{2+} channels. However, opioid receptors have not been previously associated with the phosphoinositide turnover response. We have investigated the effects of various subtype-selective opioid agonists and antagonists on PI turnover response in the rat brain. U-50,488H, a selective κ-agonist, enhanced the hydrolysis of inositol phospholipids, as reflected by increased [^{3}H]-inositol phosphates ([^{3}H]-IP's) formation in rat hippocampal slices prelabeled with [^{3}H]-inositol and treated with Li^+. The κ-selective agonists U-50,488H ketocyclazocine and D[Ala]^2-dynorphin-A (1-13) amide produced a concentration-dependent increase in the accumulation of IP's in hippocampal slices without altering the incorporation of [^{3}H] inositol into phospholipids. The EC_{50} values were 140, 23, and 16 μM, respectively. Dynorphin-A (1-13) amide also produced a significant increase in the formation of [^{3}H]-IP's. On the other hand the μ-selective agonists [D-Ala^2-N-Me-Phe^4-Gly^5-ol]-enkephalin and morphine, the δ-selective agonist [D-Pen^{2,5}]-enkephalin and etorphine were ineffective in stimulating IP's formation in hippocampal slices. The increase in IP's formation elicited by U-50,488H was partially antagonized by naloxone and more completely antagonized by the κ-selective antagonists nor-binaltrophimine and MR 2266. The formation of IP's induced by U-50,488H varies across different regions of the brain, being highest in hippocampus and amygdala, and lowest in striatum and pons-medulla. The results indicate that brain κ– but neither μ- nor δ-receptors are coupled to the PI turnover response in the brain. It is suggested that the effects of dynorphin in brain and perhaps other tissues are mediated by κ-receptors linked to the PI turnover response. Supported by DA04068.

392.10

CHANGES IN THE SUBCELLULAR DISTRIBUTION AND GTP SENSITIVITY OF OPIOID RECEPTOR BINDING IN UP-REGULATED NEUROBLASTOMA-HYBRID CELLS. M. Belcheva*, R.J. McHale+, O.Dillon-Carter*, D.-M. Chuang# and C.J. Coscia+ (Spon: M.H. Cooper). Dept. Biochem., St. Louis Univ. St. Louis, MO and #Lab. Preclin. Pharmacol., NIMH, St. Washington, DC.

Membranes from Con A-treated NCB-20 and NG108-15 cells were resolved into light (LM) and heavy (HM) fractions by sorbitol density gradient centrifugation. Marker enzyme distribution data indicated that HMs were enriched in plasma membrane whereas LMs contained most of the endoplasmic reticulum and Golgi bodies. In both cell lines, HMs constituted 80-90% and LMs 10-20% of the δ opioid receptor specific binding, as measured with ^{3}H[D-Ala^2,D-Leu^5]enkephalin (DADLE). ^{3}H-DADLE binding to HMs was inhibited to a greater extent by guanosine-5'-(βγ-imido) triphosphate (Gpp(NH)p) than that of LMs for both cell lines, in agreement with our results with adult rat brain. Up-regulation was achieved by pretreating NCB-20 cells with 1 mM Na butyrate for 3 d or NG108-15 cells with 1 μM Naltrexone for 2 d. LMs from both upregulated cell lines were elevated 2-3 fold while HMs displayed a 1-2 fold increase in ^{3}H-DADLE binding. Dose-dependent Gpp(NH)p inhibition of δ binding in up-regulated LMs was greater than in controls. HM δ binding sensitivity to Gpp(NH)p was unchanged. These results taken together with data from neonatal rat brain studies suggest that LMs from up-regulated cells contain more newly synthesized opioid receptors than controls.

392.11

REVERSIBLE LOSSES IN OPIOID RECEPTOR EXPRESSION BY NEUROBLASTOMA CELL LINE N18TG2 IN VIVO. G.E. Thomas*, W.T Bem*, M. Belcheva*, D.C. Palmer*, K.C. Tolman*, F.E. Johnson*, and C.J. Coscia (Spon: S. Horenstein). St. Louis Univ. Med. Ctr., St. Louis, MO 63104.

Opioids regulate neuroblastoma growth via receptor mediated mechanisms. A current experimental model is the murine neuroblastoma line N18TG2, but the stability of its expression of opioid receptors in vivo has not been reported. N18TG2 cells were injected into male nude mice and the tumors passaged serially. At each generation, tumors were excised at necropsy and analyzed for δ opioid receptor binding parameters using ^{3}H[D-Ala^2,D-Leu^5]enkephalin in homologous displacement assays. Kd and Bmax were determined using the LIGAND program. Bmax for cultured cells (184±35 fmol/mg protein) declined with successive transfer, reaching 0 by passage 3. Tumor cells from passage 4, which displayed no detectable opioid receptor binding, were then returned to culture medium; 6 weeks later their opioid receptors were up-regulated (Bmax, 509±82 fmol/mg protein). Light microscopy revealed no morphological differences between the original and recultured cells. Repassaging the recultured cells in vivo led again to a rapid decline in opioid receptor expression. The Kd for DADLE (2.5 nM) remained unchanged. δ Opioid receptor expression is thus a non-constitutive property of N18TG2. This alteration of receptor expression represents an important variable and may prove useful in delineating the mechanism of opioid modulation of tumor growth.

392.12

AFFINITY LABELING OF MU OPIOID RECEPTORS IN BRAIN BY BROMOACETAMIDO DERIVATIVES OF MORPHINE. J.M. Bidlack[1], D.K. Frey[1], A. Seyed-Mozaffari[2] and S. Archer[2]. [1]Dept. of Pharmacology, University of Rochester, School of Medicine and Dentistry, Rochester, NY 14642 and [2]Dept. of Chemistry, Rensselaer Polytechnic Institute, Troy, NY 12181.

After reduction of a disulfide bond at or near the μ opioid binding site in rat brain membranes, 14β-bromoacetamidomorphine (BAM) has been shown to alkylate this site, resulting in the irreversible inhibition of greater than 90% of the μ opioid binding to rat brain membranes. The analogues 14β-bromoacetamido, 7,8-dihydromorphine (H_2BAM), 14β-bromoacetamidomorphinone (BAMO) and 14β-bromoacetamido, 7,8-dihydromorphinone (H_2BAMO) were tested for their ability to alkylate the μ opioid binding site. In the absence of a reducing agent, all affinity ligands bound reversibly. Their order of potency in inhibiting the binding of the μ-selective peptide [^{3}H]DAGO was H_2BAMO > BAMO = morphine > H_2BAM > BAM. When membranes were incubated with dithiothreitol followed by one of the affinity ligands, and then extensive washing, μ opioid binding was irreversibly inhibited, while binding to δ and κ sites was not altered by the affinity ligands. Their order of potency in irreversibly inhibiting μ opioid binding was the same as their rank order under reversible binding conditions. Only opioid alkaloids and peptides that bind to μ opioid receptors protected the site from alkylation. These affinity ligands will facilitate the characterization of the μ opioid binding site. (Supported by USPHS grants DA03742 and DA01674.)

392.13

PRETREATMENT OF RATS WITH THE IRREVERSIBLE μ-RECEPTOR ANTAGONIST, β-FNA, FAILS TO PREVENT NALTREXONE-INDUCED UPREGULATION OF μ-OPIOID RECEPTORS. **R. B. Rothman[1], J. B. Long[2], V. Bykov[1], K. C. Rice[3] and J. W. Holaday[2]. [1]Unit on Receptor Studies, LCS, NIMH, Bethesda, MD 20892. [2]Neuropharmacology Branch, Walter Reed Army Institute of Research, Washington, DC 20307-5100.[3] Laboratory of Medicinal Chemistry, NIDDK, Bethesda, MD 20892.**

This study examined the effect of FNA, an irreversible μ receptor antagonist, on naltrexone-induced upregulation of μ opioid receptors. Rats were treated according to the following protocol. Day #1: two naltrexone or two placebo pellets were implanted subcutaneously in a nylon mesh. Day #8: the pellets were removed intact. Rats were administered either saline or 20 nmol of FNA in 10 μl of saline (i.c.v.) on day #1,3, 5 and 6, 60 min prior to pellet implantation. Day #9: frozen lysed-P2 membranes were prepared from whole brain (minus cerebellum) and μ binding sites were assayed using [^{3}H]FOXY (New England Nuclear, SA=40.1 Ci/mmol) as previously described. The results were:

	GROUP	Bmax	Kd
1.	Placbo/Saline	112±4.6	3.80±0.13
2.	Placebo/FNA	94±13	11.2±1.0*
3.	Naltrexone/Saline	173±3.0	2.87±0.05*
4.	Naltrexone/FNA	165±9.0	8.35±0.20*

These data demonstrate that functional blockade of μ receptors with FNA does not upregulate μ receptors or prevent naltrexone-induced upregulation.

392.14

NALTREXONE-AMPHETAMINE INTERACTIONS IN THE REGULATION OF STRIATAL PRODYNORPHIN PEPTIDES. K.A. Trujillo and H. Akil. Mental Health Research Institute, University of Michigan, Ann Arbor, MI 48109-0720.

We have previously reported that chronic treatment with either amphetamine or morphine will increase prodynorphin (PRODYN) peptides in the striatonigral system. In order to further study the regulation of this system by dopaminergic and opiate agents we examined the effects of amphetamine and naltrexone, administered either separately or together, on PRODYN peptides in forebrain and midbrain regions. Adult male Sprague-Dawley rats received a single subcutaneous injection each day for 7 days of either saline (SAL; 1ml/kg), d-amphetamine sulfate (AMPH; 5 mg/kg), naltrexone HCL (NTX; 10 mg/kg), or AMPH (5 mg/kg) and NTX (10 mg/kg) administered together. Animals were sacrificed 24 hours after the final injection and selected brain regions were radioimmunoassayed for PRODYN peptides. In dorsal striatum (caudate-putamen) AMPH caused a dramatic increase (approximately 100% increase over SAL-treated animals) in peptide content. NTX, while causing no effects alone, partially blocked the effects of AMPH in this brain region. In ventral striatum (nucleus accumbens-olfactory tubercle) AMPH caused a modest increase in peptide content. In addition, NTX caused a similar increase in this brain region. However, when AMPH and NTX were administered together, no increase in content was seen. These results suggest that PRODYN neurons in the dorsal and ventral striatum are differentially regulated by dopaminergic drugs and opiate antagonists, and demonstrate complex interactions between these drugs in the regulation of PRODYN peptides. Further studies are necessary to elucidate the mechanisms responsible for the complex interactions between these drugs in the two brain regions. Supported by NIDA NRSA DA05336 (K.A.T.) and NIDA DA02265 and NIMH MH422251 (H.A.)

392.15

DIFFERENTIAL EFFECTS OF ELECTRICAL STIMULATION IN THE MIDBRAIN PERIAQUEDUCTAL GRAY ON BETA-ENDORPHIN-ir PEPTIDE FORMS IN DIFFERENT TERMINAL FIELDS OF RAT BRAIN. D. Bronstein and H. Akil, Mental Health Research Institute, University of Michigan, Ann Arbor, MI 48109.

Proopiomelanocortin (POMC) cell bodies in the arcuate nucleus of the hypothalamus send axonal projections which innervate a diverse number of brain regions, including the septum, amygdala, periventricular nucleus of the thalamus, various hypothalamic nuclei, and the midbrain periaqueductal gray (PAG). The purpose of the present study was to examine how electrical stimulation in one POMC nerve terminal region (i.e., the PAG) might affect the content of various beta-endorphin (BE)-ir peptides in other POMC projection areas. Acute (10 min) electrical stimulation in the PAG resulted in no changes in total BE-ir content in any of the brain regions examined. However, animals chronically stimulated tended to have higher BE-ir levels in the the arcuate nucleus, dorsal hypothalamus, and septum compared to control animals. Chromatographic analyses revealed that the concentration of BE_{1-31} was increased relative to that of BE_{1-27}-size peptides in the midbrains of acutely stimulated animals; conversely, the ratio of BE_{1-31}:BE_{1-27} concentrations was decreased in the dorsal hypothalamus. The stimulation-induced alteration in the relative amounts of BE_{1-31} and BE_{1-27} may be an important indicator of the physiological tone in specific terminal fields. These results also demonstrate that electrical stimulation in one nerve terminal region has disparate effects on other BE-containing brain regions, suggesting that these effects are probably not mediated by a single mechanism. (Supported in part by NIDA #DA02265 to H.A. and a MRC Fellowship to D.B.)

392.16

MET-ENKEPHALIN-LIKE IMMUNOREACTIVITY IN PORCINE TISSUE EXTRACTS. D.L. Lucas*, J.E. Bailey*, L.D. Aimone, T.L. Yaksh, V.L.W. Go*. (SPON: B.F. Westmoreland). Mayo Clinic, Rochester, MN 55905, Univ. of CA, San Diego, LaJolla, CA 92093, and Univ. of CA, Los Angeles, CA 90024.

We have developed a radioimmunoassay for met-enkephalin (Enk) using an antibody (N382) with a crossreactivity of 31, 25, 11, and 5% to Lys6, Arg6, Arg6-Phe7, and Arg6-Gly7-Leu8 carboxy terminus extensions of Enk, respectively. Pigs (n=4) were dissected immediately postmortem into 111 brain, cord, gut and ganglia sections and extracted in 0.1 N HCl. Total encrypted Enk was also evaluated in brain regions after trypsin/carboxypeptidase B treatment of extracts, and ratios of total Enk/Enk calculated.

Region	Enk[1]	Ratio[2]	Region	Enk[1]	Ratio
Pituitary	843	2.6	Nuc Accumb	139	3.7
Glob Pal/Putamen	292	1.6	Peri Gray	105	2.9
Caudate Nucleus	162	2.1	Septum	85	4.7
Sub Nigra	149	2.5	Mam Body	52	4.1
Hypothalamus	92	3.4	Medulla	36	5.7
Cerv Dor Horn	18.4	-	Duodenum	111	-
Sacr Dor Horn	37.1	-	Colon	10.4	-

[1]Enk=ng/gm; [2]Ratio=Total Enk/Enk

The varying ratios of total Enk/Enk in selected brain regions may be related to differential processing of opioid precursors reported by others.

396.17

STRUCTURE-ACTIVITY RELATIONSHIPS OF DTG AND ITS CONGENERS AT THE HALOPERIDOL-SENSITIVE SIGMA RECEPTOR. B. Tester, M. Scherz*, M. Fialeix*, J. Keana* and E. Weber. Vollum Institute, Oregon Health Sciences University, Portland, OR 97201 and Department of Chemistry, University of Oregon, Eugene, OR 97403.

With an eye toward the full biochemical characterization of the haloperidol-sensitive sigma receptor, we have studied the structure-activity relationships of di-o-tolylguanidine (DTG) and its congeners at this site. A number of DTG analogs were synthesized and evaluated in in vitro radioligand displacement experiments with guinea pig brain membrane homogenates, using the highly sigma-specific radioligands [3H]DTG and [3H](+)3-PPP, and the PCP receptor-specific compounds [3H]TCP and [3H](+)-MK-801. The affinity of N,N'-diarylguanidines for the sigma receptor decreases with increasing steric bulk of ortho substituents larger than CH_3, and hydrophobic substituents are preferred over hydrophilic ones, regardless of their position on the ring. Furthermore, electroneutral substituents are preferred over strongly electron donating or withdrawing groups. Potent binding to the sigma receptor is retained as long as at least one side of the guanidine bears a preferred group (e.g.o-tolyl). Replacement of the aryl ring with certain saturated carbocycles (e.g.cyclohexyl, norbornyl or adamantyl) leads to a significant increase in affinity. By combining the best aromatic and best saturated carbocyclic substituents in the same molecule, we arrived at some of the most potent sigma ligands described to date, e.g. N-endo-2-nor-bornyl-N'-2-iodo-phenylguanidine (IC_{50} of 3 nM vs. [3H]DTG). All of the compounds tested were several orders of magnitude more potent at the sigma receptor than at the PCP receptor, with a few notable exceptions. This series of disubstituted guanidines is of great value in the development of potential antipsychotics, and in the further pharmacological and biochemical characterization of the sigma receptor. We thank Nam Hoang for his contribution to this project. Supported by NIMH grants MH40303 and MH42608, a grant from Cambridge NeuroScience Inc.

396.18

DYNORPHIN-SELECTIVE INHIBITION OF ADENYLATE CYCLASE IN GUINEA PIG CEREBELLUM MEMBRANES. C.S. Konkoy and S.R. Childers, Dept. of Pharmacology, Univ. Florida Coll. Med., Gainesville, FL 32610.

Kappa opioid receptors have been characterized by their binding and pharmacological properties, but the question of which second messenger systems are coupled to kappa receptors is not yet settled. Both mu and delta opioid receptors inhibit adenylate cyclase in brain membranes. Guinea pig cerebellum, which contains kappa receptors relatively uncontaminated by other opioid receptor types, was chosen to examine whether kappa receptors are coupled to adenylate cyclase. Membranes were prepared from guinea pig cerebellum and pretreated at pH 4.5 to increase inhibitory activity, and adenylate cyclase was assayed in the presence of dynorphin analogs as prototypical kappa agonists. Results showed that several dynorphin analogs inhibited adenylate cyclase by 30%-50%, whereas mu and delta preferring agonists had no effect. Dynorphin A and the kappa-selective compounds D-pro^{10}-dynorphin 1-11 and U-50,488H were the most potent agonists, with IC_{50} values of 0.03-0.05 μM, while other dynorphin gene products like dynorphin B and α-neo-endorphin were approximately ten-fold less potent. Like other G_i-coupled responses, dynorphin-inhibited adenylate cyclase required GTP and sodium. Naloxone was a competitive antagonist for dynorphin-inhibited adenylate cyclase, with 1 μM naloxone shifting the IC_{50} value of dynorphin A by 20-fold. The kappa-selective antagonist nor-binaltorphimine (nor-BNI) was even more potent, with 0.1 μM nor-BNI shifting the dynorphin IC_{50} value by 50-fold. These results suggest that dynorphin A and its analogs inhibit adenylate cyclase by binding to a G protein-coupled opioid receptor whose pharmacological specificity matches those of kappa receptors.

Supported by PHS grant DA-02904 from the National Institute on Drug Abuse.

396.19

REGULATION OF TYPE I ASTROCYTE PROENKEPHALIN: TRANSCRIPTION, TRANSLATION, AND SECRETION. K. G. Low*, R. G. Allen, C. P. Nielsen*, R. P. Saneto, S. L. Young*, and M. H. Melner* (SPON: B. V. Critchlow). Oregon Regional Primate Research Center, Beaverton, OR 97006 and Oregon Health Sciences University, Portland, OR 97201.

The expression and regulation of proenkephalin, proopiomelanocortin, and prodynorphin was investigated in primary cultures of purified glial cells from the neonatal rat cerebral cortex. Northern blot analysis revealed that of all three opioid genes, only the proenkephalin gene was expressed in type I astrocytes. No proenkephalin expression was detected in bipotential glial precursor cells or oligodendrocyte progenitor cells. Treatment of the astrocytes with 10^{-6} M isoproterenol (ISO) or 5 X 10^{-4} M chlorophenylthio-cAMP (CPT-cAMP) for 20 hours stimulated proenkephalin mRNA levels 3-fold and 6-fold, respectively. Glial fibrillary acidic protein (GFAP) mRNA levels were also stimulated by ISO or CPT-cAMP treatments by 3-fold over untreated controls. Potential transcriptional regulation of the proenkephalin promoter was examined by transfecting the fusion gene construct pENKAT-12 containing 193 bp of the 5' flanking sequence, exon I, intron A, and 53 bp of exon II of the human proenkephalin gene fused to the bacterial chloramphenicol acetyltransferase (CAT) gene into astrocytes. Treatment of the transfected cells with 10^{-6} to 10^{-8} M ISO elicited stimulations of CAT activity from 7 to 19-fold over controls. CPT-cAMP treatment stimulated CAT activity 6-fold. Immunoreactive proenkephalin was measured by radioimmunoassay using antisera against met-enkephalin-arg-phe and HPLC-separation of acetic acid extracts of cells and media. Control cultures secreted 2.3 ± 0.4 pmoles/ml. Treatment with ISO or CPT-cAMP significantly stimulated proenkephalin peptide levels to 4.0 ± 0.1 and 4.3 ± 0.3 pmoles/ml, respectively. These data suggest that type I astrocytes from neonatal rat cortex express and secrete significant levels of proenkephalin under the regulation of β-adrenergic agonists. Supported in part by NIH DK-41035, RR-00163, and ONR N00014-88-K-0030, R&T Code 441F724.

396.20

AGE-DEPENDENT SUBCELLULAR DISTRIBUTION OF OPIOID RECEPTORS AND G-PROTEINS IN RAT BRAIN. C.J. Coscia, S.J. Yeung, M. Belcheva and W.T. Bem. Dept. of Biochem., St. Louis Univ. Med. Ctr., St. Louis, MO 63104.

Evidence for ontogenic differences in binding and structural properties of rat brain opioid receptors has been reported previously. In the present study we investigated the possibility that early neonatal membranes are enriched in newly synthesized μ opioid receptors as detected with ^{3}H[D-ala^2,mePhe^4,gly-ol^5] enkephalin. Membranes from 1-day-old neonatal (P-1) and adult rat forebrain were resolved into light (LM) and heavy (HM) populations. Marker enzyme distribution indicated that LMs were enriched in endoplasmic reticulum and Golgi apparatus, while HM contained most of the plasma membranes. P-1 LMs contained 43% of the total μ-opioid receptor binding compared to 16% in their adult counterpart. Sodium ions inhibited μ-opioid binding in LMs to a greater extent (65-71%) than in HMs (45-51%) but no significant differences were seen between P-1 and adults. P-1 LM binding displayed greater sensitivity to the GTP analog, Gpp(NH)p, and LMs possessed more G-protein α-subunit than adult LMs, as shown by quantitative immunoblotting experiments with anti-G peptide antibodies. The presence of a greater proportion of different intracellular sites may be responsible for some of the previously documented differences between adult and neonatal opioid binding.

392.21

CHARACTERIZATION OF [^{3}H](+)-PENTAZOCINE, A HIGHLY SELECTIVE SIGMA LIGAND. W.D. Bowen[1], B. DeCosta[2*], S.B. Hellewell[1*], A. Thurkauf[2*], K.C. Rice[2], and J.M. Walker[3]. [1]Div. Biology and Med. and [3]Dept. Psychology, Brown Univ., Providence, RI. 02912, and [2]Lab. Med. Chem., NIDDK, Bethesda, MD 20892.

(+)-Pentazocine appears to be a selective sigma ligand, and it binds to guinea pig brain sigma receptors with an apparent K_i of 1.2 nM. This compares to a K_i of 5,530 nM at phencyclidine receptors. By comparison (+)-SKF 10,047 exibits a sigma K_i of 62.2 nM and a PCP receptor K_i of 208 nM. We surmised, therefore, that [^{3}H](+)-pentazocine would be a more selective benzomorphan-based probe for sigma receptors than the currently available [^{3}H](+)-SKF 10,047. [^{3}H](+)-Pentazocine (26.6 Ci/mmol) was synthesized by tritiation of (+)-normetazocine, followed by attachment of the prenyl side chain. Scatchard analysis in guinea pig brain membranes yielded linear plots with a K_d of 5.1 nM and a B_{max} of 1,146 fmol/mg protein. Percent specific binding was greater than 90% at concentrations up to 25 nM. Competition of various unlabeled ligands against 3 nM [^{3}H](+)-pentazocine revealed the following order of potency: haloperidol = (+)-pentazocine > DTG = (+)-3-PPP > (+)-SKF 10,047 > (-)-pentazocine >> PCP. MK-801 and apomorphine were inactive. Thus, [^{3}H](+)-pentazocine exhibits high affinity and selectivity for sigma receptors.

391.22

EVIDENCE FOR MUTUAL INTERACTIONS OF ENKEPHALIN AND CCK ANALOGUES AT CCK AND ENKEPHALIN RECEPTORS R.B. Murphy, M. Davila-Garcia, E.C. Azmitia, P. Psyllas, G. Haraminian, A. Cann, M.R. Pincus, J. Chen, L.H. Schneider, J. Gibbs, and G.P. Smith. Depts Chem. and Biol., N.Y.U., N.Y. 10003 ; Dept. Psychiatry, Bourne Lab., NYH-CUMC, White Plains, NY 10605 ; Dept of Pathol., SUNY-Hlth. Sci. Ctr., Syracuse, NY 13210

We previously predicted interactions between enkephalin (ENK) and CCK at the receptor level, based upon molecular modeling (Pincus et al, Peptides 9: S145, 1988). We here present additional experimental evidence in support of this hypothesis. Unsulfated CCK (DS-CCK-8) significantly inhibits outgrowth of fetal rat hindbrain 5-HT neurons in culture, as quantified by [^{3}H]-5-HT uptake and morphometry. Leu-ENK produces a similar result (Davila-Garcia and Azmitia, Brain Res. in press, 1989). The DS-CCK inhibition is blocked by naloxone, but not by the CCK antagonist phenoxyacetylproglumide. In another series of experiments, we examined the effect of Met-ENK upon physiological response (contraction) produced by sulfated CCK-8 in the rat pyloric sphincter, a clean peripheral-type CCK receptor preparation (Murphy et al, Peptides 8: 127, 1987). Met-ENK antagonizes the CCK-8-induced contraction (IC_{50} = 110 nM); naloxone (10 uM) is without effect. These results further support the presence of receptor-level CCK-ENK interaction. Supported by NSF BNS8812892, NIMH RO1 40010, and Hoffman-La-Roche.

391.23

CHRONIC NALTREXONE INCREASES EXPRESSION OF PREPROENKEPHALIN AND PREPROTACHYKININ mRNA IN DISCRETE BRAIN REGIONS. R.S. Zukin, J.A. Kessler and A. Tempel (SPON: A.B. Johnson). Dept. of Neuroscience, Albert Einstein College of Medicine, Bronx, NY 10461.

Long-term blockade of opioid receptors by the antagonist naltrexone increases met-enkephalin in the striatum and nucleus accumbens (Tempel et al., 1984). To determine whether these changes reflect increased peptide synthesis, we examined preproenkephalin (PPE) mRNA content in brain regions of control (placebo-treated) and naltrexone-treated animals by Northern analysis. Naltrexone treatment (8 days) led to an 11-fold increase in striatal PPE mRNA. No statistically significant change was observed in striatal cyclophilin (1B15) or actin mRNA. Small increases in PPE mRNA occurred in the hippocampus (+40%) and hypothalamus (+19%). Increases in mRNA occurred 24 hrs following onset of antagonist; increases in opioid receptors were half maximal 3-4 days after onset of antagonist. To determine whether substance P synthesis is altered by naltrexone, preprotachykinin (PPT) mRNA content was examined. Naltrexone (8 days) increases striatal PPT mRNA by 5-fold. Striatal substance P was increased 3-fold. These findings suggest that chronic blockade of brain opioid receptors leads to the increased synthesis of both enkephalin and substance P in the striatum, and that the changes are relatively specific. The finding of opiate antagonist-induced increases in PPT mRNA and substance P supports the concept of a role for enkephalin in the regulation of substance P gene expression. [Supported by NIH grants DA04439 (R.S.Z.); DA05440 (A.T.) and NS20778 (J.A.K.)].

mRNA REGULATION IV

393.1

ESTRADIOL REGULATION OF ESTROGEN RECEPTOR mRNA IN RAT HYPOTHALAMUS. A.H. Lauber, G.J. Romano, C.V. Mobbs, P. Chambon and D.W. Pfaff. Lab of Neurobiology and Behavior, Rockefeller University, New York, NY 10021.

In situ hybridization was used to investigate estradiol regulation of estrogen receptor (ER) mRNA. Ovariectomized (Ovx) rats were treated with 10 ug estradiol benzoate (EB) for 0,2,4,18 or 24 hrs. Brain sections were hybridized with a 3H-single-stranded DNA probe prepared from the pORF cDNA of the human ER gene and exposed to nuclear emulsion for 4 months. Specificity of labelling was determined by counting grains/cell in ventromedial nucleus of the hypothalamus (VLVM) compared to cells in thalamus and cortex, and by comparing to sections pretreated with RNAse or hybridized with a non-sense probe. Labelling for ER mRNA was not apparent in regions known to contain high concentrations of glucocoorticoid and thyroid receptor mRNAs. ER-expressing cells constituted 30-40% of cells in arcuate nucleus and VLVM, respectively, in close accordance with data from ER steroid autoradiography (Exp. Brain Res. 62:343). Highest levels of ER mRNA were present in the Ovx controls. ER mRNA levels were decreased 2 hrs following EB administration, fell 42% (VLVM) and 64% (arcuate) by 18 hrs, and remained depressed at 24 hrs. These data show that estrogen can down-regulate ER mRNA in neurons implicated in different estrogen-dependent functions, and as reported for ER mRNA in MCF-7 cells.

393.2

REGULATION OF THE GLUCOCORTICOID SENSITIVITY OF NEUROBLASTOMA AND MOUSE L CELLS BY TRANSIENT EXPRESSION OF A GLUCOCORTICOID RECEPTOR (GR) ANTI-SENSE RNA AND BY DESIPRAMINE. Marie-Claude Pepin* and Nicholas Barden. Ontogénèse et Génétique Moléculaires, Le Centre de Recherche du Centre Hospitalier de l'Université Laval, Ste-Foy, Québec G1V 4G2, Canada.

Depression is often characterized by increased cortisol secretion caused by hyperactivity of the hypothalamo-pituitary-adrenal (HPA) axis and by non-suppression of cortisol secretion following dexamethasone administration. This hyperactivity of the HPA axis might be caused by a reduction of GR in brain cells involved in the control of the axis. This hypothesis is in agreement with our results which show that antidepressants may normalize the HPA axis by increasing neuronal GR mRNA levels (PEPIN et al. Mol. Brain Res., in press.) In order to investigate the effect of reduced neuronal GR levels, we have blocked cellular GR mRNA translation by introduction of a complementary GR anti-sense RNA strand. Two cell lines, mouse LTK$^-$ cells and neuroblastoma NB41A3 cells were transfected with a reporter plasmid carrying the chloramphenicol-acetyl-transferase (CAT) gene under MMTV-LTR (a glucocorticoid inducible promotor) control. This gene construction permitted assay of the sensitivity of the cells to glucocorticoid hormones. Cells were also co-transfected with a plasmid containing 1800bp of GR cDNA in the reverse orientation downstream from a neurofilament gene promotor element and the sensitivity of CAT activity to exogenous dexamethasone subsequently measured. While, in control incubations, dexamethasone increased CAT activity by as much as 13 fold, expression of GR anti-sense RNA caused a 2-4 fold decrease in the CAT response to dexamethasone.When cells were incubated in the presence of antidepressant (desipramine), a two to three fold increase in the sensitivity of CAT activity to dexamethasone was seen. These results validate the use of antisense RNA to GR in order to decrease the cells response to glucocorticoids and confirm the antidepressant-induced modulation of cell sensitivity to glucocorticoids.

393.3

DISTRIBUTION OF CHOLINE ACETYLTRANSFERASE mRNA IN THE RAT BRAIN. M.V. Lorenzi*, D.C. Hilt, L.B. Hersh, C.F. Kong*, F. Hefti, and W.L. Strauss (SPON: L.T. Potter). Dept. of Pharmacology, Univ. of Miami, Dept. of Neurology, Univ. of MD School of Med., Dept. of Biochemistry, Southwestern Med. Ctr. at Dallas and Andrus Gerontology Ctr. U.S.C.

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

393.4

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

We have quantitated GAD mRNA levels in the different GABAergic cell types of the cerebellum and compared these to the levels of intracellular poly(A). In normal rat and mouse, all Purkinje cells contain GAD mRNAs, although the amount in individual cells fluctuates. The relative amount of GAD mRNA in different cell types does not differ more than 3 fold. In adult nervous mouse cerebellum, > 90% of Purkinje cells have degenerated. The remaining Purkinje cells have approximately the same levels of GAD mRNA as those in normal mice. GAD mRNA levels are also still high in the other GABAergic cell types. In developing rat cerebellum, GAD mRNA levels in the prenatally-formed Purkinje and Golgi cells is already high by P14. No GAD mRNAs are seen in the EGL and they are infrequent in the proximal parts of the molecular layer. GAD mRNA in stellate and basket cells increases as the cells migrate towards the IGL. These results show that GAD mRNA levels in Purkinje cells do not fluctuate greatly, either under normal physiological conditions or when Purkinje cell number is greatly depleted. Developmentally, GAD mRNAs are rapidly induced in Purkinje and Golgi cells and are induced in stellate amd basket cells as they migrate through the molecular layer. Supported by NIH 14886 (MRM-B) and Zale Foundation (RNR).

393.5

GABA RECEPTOR MODULATION OF TYROSINE HYDROXYLASE GENE EXPRESSION IN THE ADRENAL GLAND C. Hale*, M. Wessels-Reiker*, M.A. Moore*, and R. Strong. Geriatric Research, Education and Clinical Center, St. Louis VA Medical Center and St. Louis University School of Medicine, St. Louis, MO 63125

Several studies demonstrated the presence of GABA receptors on chromaffin cells of the adrenal medulla. Furthermore, other studies suggest that GABA receptors in the adrenal gland are important regulators of catecholamine release from chromaffin cells. For example, bicuculine has been shown to increase the release of catecholamines elicited by splanchnic nerve stimulation. Since those stimuli which increase catecholamine release from the adrenal often induce TH gene expression, we examined the role of GABA receptors in plasticity of TH gene expression.

We treated animals with a single injection of reserpine (7.5 mg./Kg./s.c.), or the GABA antagonist bicuculine (10mg/Kg/s.c.) twice a day for three days, or a combination of a single reserpine treatment and treatment with bicuculine for three days. At the end of three days the animals were sacrificed and the adrenal glands and brains were rapidly removed for assays of TH activity and TH mRNA content. Bicuculine treatment for three days had no effect on TH activity or TH mRNA. Reserpine treatment had the expected effect of increasing TH mRNA and TH activity by 2 fold. However, the combination of bicuculine and reserpine significantly increased TH activity and TH mRNA content as compared to the reserpine group. These data suggest a role for GABA receptors in regulation of TH mRNA in the adrenal gland.

393.6

STUDIES ON GENE EXPRESSION OF TYROSINE HYDROXYLASE (TH) IN ADRENAL GLANDS OF SHR AND WKY RATS. H. Zawadzka*, S. Tsuda*, D. Filer*, P. Benedetto*, W. Chan* and M. Goldstein. Neurochem.Res. Lab, N.Y. Univ. Med. Ctr., New York, NY 10016.

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

393.7

HUMAN DOPAMINE BETA-HYDROXYLASE (DBH): IN VITRO TRANSCRIPTION AND TRANSLATION OF ENZYMATICALLY AMPLIFIED cDNA. C. M. Craft, T. H. Thai*, R. Gonzalez*, and J. D. Raese. Lab. of Molecular Neurogenetics, Schizophrenia Research Ctr., VA Med. Ctr. and Dept. of Psychiatry, Univ.TX Southwestern Med.Sch., Dallas, TX 75216.

The 5'coding region of human DBH was cloned using the polymerase chain reaction (PCR) and sequenced by dideoxysequencing. The predicted amino terminus of DBH was MetArgGluAla (MREA), in agreement with Lamouroux et al.(EMBO J. 6:3931, 1987). We discovered that this sequence is homologous to the amino terminus of beta tubulin (MREI) which mediates autoregulation of tubulin mRNA stability by free tubulin subunits (Yen et al.,Nature 334: 580, 1989). Mutagenesis of tubulin codon 2 (R) or codons 3 and 4 (EI) abolishes autoregulation of tubulin mRNA stability. A search of the Swiss Protein Data Base revealed no other mammalian protein with an amino terminal MRE{A,I}. We propose that the nascent amino terminus of DBH may interact with unassembled tubulin subunits and regulate DBH mRNA stability. Coupling of DBH synthesis and exocytosis could be accomplished through changes in the concentration of unassembled tubulin subunits.

To test this hypothesis, we amplified DBH cDNA of increasing size starting with the predicted initiation codon (AUG) using PCR. Chimeric oligonucleotides encoding a T7 RNA polymerase promoter and the 5'region of DBH were used in the amplification of cDNA. The PCR products of the predicted sizes were detected on 3% agarose gels and gel purified fragments were sequenced to verify their identity. Transcripts of these DBH templates were synthesized with T7 RNA polymerase and translated in vitro with a reticulocyte lysate system. This new PCR/transcription/translation system is being utilized to study the potential translational regulation of the nascent amino terminus of DBH by unassembled tubulin subunits.

393.8

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

The role of neurotransmitters and receptors in the reserpine elicited increases in TH mRNA and TH enzyme in the locus ceruleus is unknown. Furthermore, the neurochemical nature of many of the afferents to locus ceruleus is not completely known. In order to determine the relationship between locus ceruleus afferents and TH gene expression in response to reserpine, we mapped the topography of TH activity, TH mRNA and neurochemical markers of the cholinergic and GABAergic systems.

Rats were treated with a single injection of reserpine (7.5 mg./Kg./s.c.). or vehicle. Three days after injection, the animals were sacrificed and the brain rapidly removed and frozen in powdered dry ice. Brains were cut through the length of the locus ceruleus using a freezing microtome. Alternating 300 μm and 15μm sections were obtained. Tyrosine hydroxylase, glutamic acid decarboxylase and choline acetyltransferase were measured in 1mm punches from the locus ceruleus in three successive 300 μm thick sections. TH mRNA was measured by in situ hybridization in the thin sections, using a 48 oligonucleotide synthetic cDNA probe.

TH mRNA levels differed between subregions of the locus ceruleus. After reserpine, TH enzyme and TH mRNA increased, but only in selected subregions of the LC. The regionally selective change in TH and TH mRNA suggests that those neurotransmitters that are highest in the affected regions play a major role in regulation of TH gene expression.

393.9

CHRONIC ANTIDEPRESSANT REGULATION OF c-FOS EXPRESSION IN RAT CEREBRAL CORTEX. S.M. Winston*, M.D. Hayward*, E.J. Nestler, and R.S. Duman. Laboratory of Molecular Psychiatry, Dept. of Psychiatry, Yale University School of Medicine, New Haven, CT 06508.

Acute seizures and other stimuli cause a rapid induction in neurons of the nuclear protein c-fos, an effect which may underlie some long-term adaptive responses to acute neuronal activation. In the present study, we examined the influence of chronic electroconvulsive seizures (ECS) on the regulation of c-fos induction in rat cerebral cortex. We found that 4 hr after acute ECS, the induction of c-fos mRNA by a second ECS is blocked, in agreement with earlier findings, but, by 1 d, c-fos mRNA could be re-induced by a second ECS, indicating that the responsiveness of this system can be fully "reset" after 1 d. However, when rats received chronic (daily) ECS, c-fos induction in response to the most recent ECS showed a time-dependent decrease. This effect was maximal after 7-10 d of chronic ECS, when the induction of c-fos mRNA, as well as of Fos protein, by the most recent seizure was completely blocked. Chronic ECS similarly blocked the induction of c-jun, another nuclear protein. The blockade of c-fos induction observed 4 hr after acute ECS and after chronic ECS may involve different mechanisms, since it is known that Fos protein remains elevated 4 hr after acute seizure, but is at control levels after chronic ECS.

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

393.10

CHRONIC ANTIDEPRESSANT ADMINISTRATION DECREASES THE EXPRESSION OF TYROSINE HYDROXYLASE IN RAT LOCUS COERULEUS. A. McMahon, E.L. Sabban, J.F. Tallman, E.J. Nestler and R.S. Duman. Depts. Biochem. and Mol. Biol., NY Med. Col., Valhalla, NY 10595 and Lab. Mol. Psychiatry, Yale Univ. Sch. Med., New Haven, CT 06508.

Chronic administration of many types of antidepressants down regulates the postsynaptic β-adrenergic receptor system in cerebral cortex. In the present study, we examined the influence of chronic antidepressant treatment on the expression of tyrosine hydroxylase (TH) in the locus coeruleus (LC), the major noradrenergic nucleus in brain, to assess the presynaptic state of the noradrenergic system. Rats were treated chronically with imipramine (IMI), tranylcypromine (TCP), electroconvulsive seizures (ECS) or fluvoxamine (FLU), and levels of TH immunoreactivity or mRNA were determined in micro-punches of LC and substantia nigra (SN, a dopaminergic nucleus). All 4 treatments resulted in a 50 to 65% decrease in TH immunoreactivity in the LC, while TH levels in the SN were not altered. Moreover, chronic administration of either IMI or TCP (FLU and ECS were not tested) decreased the levels of TH mRNA by 50% in the LC, but not SN, while dopamine β-hydroxylase mRNA levels were not altered in the LC.

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

393.11

INCREASING CELL-CELL CONTACT IN THE RAT PHEOCHROMOCYTOMA CELL LINE PC18 INCREASES TYROSINE HYDROXYLASE m-RNA LEVELS AND TRANSCRIPTION OF THE TYROSINE HYDROXYLASE GENE. C.D. Carlson*, A.W. Tank (SPON: S.A. Signs), Department of Pharmacology, University of Rochester, Rochester, NY 14642.

Cell-cell contact regulates the levels of tyrosine hydroxylase (TH) in bovine adrenal chromaffin cells and in rat pheochromocytoma cells. When rat pheochromocytoma PC18 cells are cultured at high density (HD) (2 x 10^5 cells/cm^2), TH activity increases over that observed in cells cultured at low density (LD) (1 x 10^4 cells/cm^2). The levels of TH-mRNA were determined by dot blot hybridization of total cellular RNA using a ^{32}P-labeled cDNA for TH-mRNA (pTH.3). After 24 hrs the levels of TH-mRNA in HD cells are 3-fold higher than those observed in LD cells; after 4 days TH-mRNA levels are 6-fold higher in HD cells. When cells are first cultured at HD and then recultured at LD, the elevated levels of TH-mRNA decrease rapidly, reaching baseline levels in 24 hrs. The HD-dependent increases in TH-mRNA may be due to an increase in the transcription of the TH gene or from a stabilization of the TH-mRNA. The relative transcription rate of the TH gene was measured with an in vitro "run on" assay using nuclei isolated from HD and LD cells. Our results indicate that the elevated levels of TH-mRNA in HD PC18 cells can be accounted for in part by an increase in the relative transcription rate of the TH gene.

393.12

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

The inherited autosomal recessive mutations, tottering (gene symbol: tg) and leaner (gene symbol: tg^{la}), are functional alleles of a single gene locus in mice. The tottering mouse mutant exhibits spontaneous absense seizures, focal motor seizures and mild hindlimb ataxia while the congenic mouse mutant leaner displays severe ataxia and no focal motor seizures. Because noradrenergic hyperinnervation of the cerebellum, hippocampus and thalamus has been observed in tottering mice (Levitt & Noebels, PNAS, 78:4630, 1981), tyrosine hydroxylase (TH) mRNA expression was examined by in situ hybridization in 12-14 week old tottering and leaner mouse mutants and age-matched heterozygous littermates. Hybridization of the ^{35}S-cRNA TH probe was detected in the major brain catecholaminergic nuclei, the locus coeruleus and substantia nigra, in control mice as well as tottering and leaner mice. Surprisingly, robust TH hybridization was observed in the cerebellar Purkinje cells of the tottering and leaner mouse mutants. Generally, cerebellar TH hybridization in tottering and leaner mice was restricted to clusters of Purkinje cells in the posterior region of the cerebellum. There was no detectable TH message in any cells of the control mouse cerebellum. Northern blot analysis confirmed these results; TH message was detected in the size fractionated cerebellar RNA of tottering and leaner mice, while little TH message was detected in control mouse cerebellar RNA. The presence of TH in the Purkinje cells of tottering and leaner mice may, in part, account for the ataxia exhibited in these mutants. These results suggest that the tg gene may play a role in the normal development of neuronal phenotypes including the assignment and maintanence of appropriate neurotransmitters. Supported by PHS NS23038.

393.13

THE EFFECT OF AGING ON THE RELATIVE mRNA CONCENTRATIONS OF MONOAMINE OXIDASE A AND B IN HUMAN BRAIN. Li-Jia Wang[1]*, Nancy Lan[1]*, Rachael Neve[2], and Jean C. Shih[1]. (SPON: J. Rho) School of Pharmacy, Univ. of Southern California, Los Angeles, CA 90033 and [2]Division of Genetics, The Children's Hospital - Boston, MA 02115

Based on substrate and inhibitor specificities, two types of monoamine oxidase (MAO), A and B, have been defined. Our recent cloning of the genes encoding MAO A and B suggest that the different enzymatic activities reside in their primary structures. By using subfragments of these cDNAs which are specific for MAO A or B, we found that MAO A and B exhibit similar patterns of expression in 14 brain regions examined; the relative concentrations of these transcripts are frontal cortex > locus coeruleus > temporal cortex > posterior pensylvian cortex-supra marginal gyri > anterior pensylvian cortex-opercular gyri ··>> hippocampus and thalamus.

Consistent with previous findings, MAO B catalytic activity increased with age whereas MAO A activity did not change. Interestingly, there is no statistical difference in mRNA levels of MAO A or B in the same aging brain samples examined. These data suggest that the increase in MAO B catalytic activity is not at the transcription level. Instead, it may be due to altered translation or post-translational modification of MAO B in aging brains. (Supported by MH39085, MH00796, and Welin professorship)

393.14

TWO MONOAMINE OXIDASE A mRNA SPECIES ARISE FROM SIZE HETEROGENIETY AT THEIR 3' TERMINI. J.-K.Huang[+], C. Huang*, L. Wen[+] and R.H. Huang, Univ. of Missouri-Kansas City, Sch. of Basic Life Sci., Kansas City, MO 64110-2499; [+]Kansas State Univ. Dept. of Biochem., Manhattan, KS 66506.

Monoamine oxidase (MAO, EC 1.4.3.4), an integral membrane enzyme, is involved in the biological inactivation of at least three different transmitter substances: dopamine; norepinephrine; and serotonin, intracellularly, in the brain. Monoamine oxidase A (MAO A) mRNA present in human placenta is comprised of two species with approximate molecular size of 2.2 and 4.2 kilobases (Kb). A cDNA clone (pMAO-A1) encoding human MAO A was isolated from a human placenta cDNA library. pMAO-A1 was 2731 base pairs long and was used to determine the molecular nature for the size heterogeneity of these mRNAs. pMAO-A1 differed from the previously published MAO A cDNA clone (Bach et al., 1988, PNAS, USA,85:4934) at their 3' termini. DNA sequencing indicated that pMAO-A1 contained an additional 1900 nucleotides at the 3' non-coding region. That pMAO-A1 corresponded to the larger mRNA (4.2 Kb) was confirmed by hybridization of a unique Hind III/EcoR1 fragment 3' to the first polyadenylational signal only to the 4.2 Kb MAO A mRNA. The result suggests that the 2.2 and 4.2 Kb mRNA species arise from the alternate use of different polyadenlylation signals. We have further isolated and characterized a MAO A genomic clone which included both the coding and non-coding regions. Southern genomic analysis suggested a single gene may encode MAO A. (Supported by grants from NIAAA and DOD to C.H. and R.H.H.)

393.15

TYROSINE HYDROXYLASE AND CHOLECYSTOKININ mRNA LEVELS IN THE SUBSTANTIA NIGRA, VENTRAL TEGMENTAL AREA, AND LOCUS CERULEUS ARE UNAFFECTED BY ACUTE AND CHRONIC HALOPERIDOL ADMINISTRATION. SL Cottingham, D Pickar*, P Montpied*, TK Shimotake*, SM Paul*, and JN Crawley. NSB, NIMH, Bethesda, MD 20892.

It is postulated that the pharmacological effects of haloperidol are mediated through dopaminergic neurons in the substantia nigra (SN) and ventral tegmental area (VTA). Cholecystokinin (CCK) coexists with dopamine in many of these neurons. To determine whether haloperidol affects tyrosine hydroxylase (TH) and CCK at the level of transcription, mRNA levels of TH, the rate-limiting enzyme in catecholamine synthesis, and of CCK, were studied.

Rats were treated with haloperidol (2 X 2 mg/kg i.p. daily) or vehicle for 3 days (acute) or 19 days (chronic) and sacrificed 16 hours after the last dose. In a separate experiment, reserpine (10 mg/kg i.p.) or vehicle was administered in a single dose 24 hours before sacrifice. Brains were frozen-sectioned at 12 µM through the SN-VTA and LC. In situ hybridization was performed using ^{35}S-labeled 48-mer oligonucleotide probes against TH and CCK (gift from W. Scott Young, NIMH). Labeled sections were exposed to Kodak XAR X-ray filmand quantitated on an image analysis system.

There was no difference in TH mRNA between control, acute haloperidol, and chronic haloperidol treatments in either the anterior, middle, or posterior VTA. There was no difference in TH mRNA in the SN between the three treatment groups. Similarly, there was no difference in CCK mRNA between control, acute haloperidol, and chronic haloperidol treatments in the anterior, middle, or posterior VTA. There was no difference in CCK mRNA in the SN between the three groups. TH mRNA was analyzed for the LC. There was no difference in mRNA levels between control, acute, and chronic haloperidol groups, although reserpine treatment produced a significant increase in TH mRNA levels in the locus ceruleus ($t = 5.938$, $p < .01$).

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

393.16

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

Galanin (GAL), a 29 amino acid neuropeptide, coexists extensively with norepinephrine (NE) in perikarya of the locus coeruleus (LC; Melander, et al. 1986). GAL has been shown to inhibit NE-induced accumulation of cyclic AMP in rat cortical slices (Nishibori, et al. 1988). Previous studies have documented that activation of LC neurons either pharmacologically or by stressors increases tyrosine hydroxylase (TH) activity (Zigmond, et al. 1974) and TH mRNA (Mallet, et al. 1983). Utilizing in situ hybridization, we have examined the effects of reserpine on both TH and GAL mRNAs in adjacent sections of the LC. Male Sprague Dawley rats were administered with either vehicle or reserpine (2 or 10 mg/kg, i.p.) and sacrificed 24 hrs later. ^{35}S oligonucleotide probes for TH (48 bases) or GAL (39 bases), synthesized by B. Martin, NIMH, were applied to coronal brain sections (12 um) which were incubated for 24 hrs. Kodak XAR X-ray film exposed by the labeled sections was quantitated using a computerized densitometry image analysis system (W. Rasband, NIH). Reserpine significantly increased both TH and GAL mRNA in LC neurons. These results confirm previous reports that reserpine increases TH mRNA concentrations in the LC. In addition, the finding that reserpine also increases GAL mRNA raises the possibility that a common reserpine-sensitive mechanism regulates the expression of both the tyrosine hydroxylase and galanin genes.

393.17

STIMULATION OF PROTEIN KINASE C INCREASES EXPRESSION OF TH AND PNMT GENES IN CULTURED BOVINE ADRENAL MEDULLARY CELLS. J.S. Hong, R. Tuominen, B.B. Kaplan, A. Poisner and M.K. Stachowiak. LMIN, NIEHS/NIH, Research Triangle Pk., NC 27709, Univ. of Pittsburgh, Pittsburgh, PA 15260, Barrow's Neurol. Inst., Phoenix, AZ 85013.

We have previously demonstrated regulation of TH and PNMT genes by cyclic AMP in cultured adrenal medullary cells (AM). The analysis of the promotors of bovine TH and PNMT genes reveals sequences homologous to the phorbol regulatory elements, suggesting regulation by protein kinase C (PKC). In this study we examined whether expression of TH and PNMT genes is related to the activity of PKC in bovine AM cells. Incubation of AM cells with 0.1 uM TPA increased membrane-bound and reduced soluble activity of PKC and induced time-dependent increases in TH and PNMT mRNA levels. Effects of TPA were reproduced with 4 β-Phorbol didecanoate known to stimulate PKC activity, but not with its inactive 4 α-analog. Twenty-four hr preincubation with 1 uM TPA down-regulated total cellular activity of PKC by 80%, and attenuated increases in mRNA levels produced by subsequent incubation with phorbol ester. Effects of TPA were also reduced by sphingosine, an inhibitor of PKC. Thus, the expression of TH and PNMT genes is regulated by PKC. The association of PKC activity with membranes may underlay these effects.

PEPTIDES: RECEPTORS IV

394.1

COMPARISON BETWEEN BINDING OF 3[H]-PROPIONYL-NEUROPEPTIDE Y IN RAT AND HUMAN FRONTAL CORTEX MEMBRANES. P.S. Widdowson* and A.E. Halaris. Dept. of Psychiatry, Case Western Res. Univ, Cleveland, OH 44109.

Neuropeptide Y (NPY), a 36 amino acid peptide shows structural similarities with members of the pancreatic polypeptide (PP) family. It exists in large quantities in mammalian brains including humans, with high levels in cortex, hypothalamus and limbic areas. We compared NPY binding sites in human and rat brain using equilibrium and kinetic studies. Human frontal cortex was frozen on dry ice and stored at -70° C. ^{3}[H]-NPY binding was performed in Krebs-Ringer buffer at 37 ° C and the bound separated from unbound by rapid centrifugation (Unden, A. et al. Eur. J. Biochem., 145: 525, 1984). Bound ^{3}[H]-NPY was counted by liquid scintillation.

^{3}[H]NPY (0.05 - 1.0 nM) bound with a high affinity to both rat and human membranes which was sensitive to GTP and magnesium. Scatchard plots of the binding showed one binding site for both rat (Kd = 0.39±0.06 nM) and human (Kd = 0.33±0.08 nM). Displacement studies showed that peptide YY (Ki= 1.36 nM: rat, Ki= 1.45 nM: human) had a similar potency to NPY (Ki= 0.49 nM: rat, Ki= 0.43 nM: human) in its ability to displace the labelled peptide whilst human and rat PP were unable to displace ^{3}[H]-NPY at 10^{-6}M. Kinetic studies showed that rat and human cortex had similar association and dissociation constants. No evidence for multiple binding sites for NPY were found in either rat or human.

394.2

STRUCTURE-AFFINITY STUDY OF NEUROPEPTIDE Y ANALOGS IN THE RAT BRAIN. J.C. Martel[1], A. Fournier[2], M. Forest[*2], S. St-Pierre[*2] and R. Quirion[1]. (1) Depts. Psychiatry and Pharmacology and Douglas Hospital Research Center, McGill University, Montreal, QC and (2) INRS Santé, Pointe-Claire, QC.

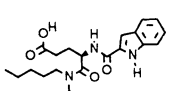
Neuropeptide Y (NPY) is a 36 amino acid peptide originally isolated from porcine brain (Tatemoto et al., PNAS 79: 5485, 1982). Various behavioral effects of centrally administered NPY have been reported. However, the physiological role(s) of this peptide is most difficult to determine because of the lack of selective, potent and stable receptor agonists and antagonists. The present study was undertaken in order to better define the structural requirements of the brain NPY receptor. The effects of successive substitutions of the first 10 amino acid residues by alanine and of tyrosine residues in position 20 and 21 was therefore studied. The affinity of these analogs for central NPY receptors was evaluated as described before for a serie of NPY fragments (Martel et al., Peptides 7: 55, 1986). All substitutions in position 1 to 10 decreased the affinity of the analog for central NPY receptors, that of the proline residue in position 5 inducing the most marked deficit. Most substitutions of the tyrosine residue in position 20 also had major effects on the affinity for the NPY binding sites while modifications in position 21 were less critical. For example, substitution in position 20 by a phenylalanine reduced the affinity to 6 % as compared to NPY while the same modification in position 21 had no effect. These data are useful in order to determine which position of the NPY molecule is required to maintain receptor affinity.

(Supported by MRCC).

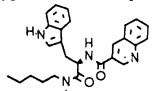
394.3

NOVEL CHOLECYSTOKININ ANTAGONISTS OF HIGH AFFINITY AND SELECTIVITY FOR CCK TYPE A RECEPTORS.
J. F. Kerwin, Jr.*, F. Wagenaar*, H. Kopecka*, C. W. Lin*, T. Miller*, D. Witte*, A. M. Nadzan*, (SPON: R. S. Janicki), Neuroscience Research Division, Pharmaceutical Discovery D-47H, Abbott Laboratories, Abbott Park, Illinois 60064.

Recently we reported on the intriguing structural similarities of glutamic acid based CCK antagonists (CR-1409) and the benzodiazepine CCK antagonist L-364,718. In our search for novel CCK receptor antagonists we have attempted to include the weak CCK antagonist benzotript into our general construct of CCK antagonists. Utilizing a similar approach to that which enabled the development of potent and selective glutamic acid based hybrid antagonists (A-64,718, A-65,186), we have obtained novel CCK antagonist hybrids of benzotript with high affinity and selectivity for type A CCK receptors. We will report on our approach in the discovery of these compounds and provide preliminary *in vitro* chacterization of these derivatives. A prototypical example of this class is A-67,396 (IC_{50}= 30 nM). With these new agents it may be possible to further probe the peripheral type A CCK receptor.



A-64,718



A-67,396

394.4

DEVELOPMENT OF A BIOTINYLATED PROBE FOR INSULIN-LIKE GROWTH FACTOR-I (IGF-I) RECEPTORS USING A NOVEL DOT BLOT ASSAY WITH NEUROBLASTOMA CELLS.
M.G. King, T.H. Wimpy*, and D.G. Baskin. Depts. Biological Structure and Medicine, University of Washington, Seattle, WA 98195, and V.A. Med. Center, Seattle, WA 98108.

We have previously localized IGF-I receptors in the brain and retina using quantitative autoradiography (QAR). With the aim of identifying cells with IGF-I receptors, we evaluated the binding of biotinylated IGF-I (b-IGF-I) to rat B104 neuroblastoma cells. B104 cells were blotted (50,000 cells per dot) on nitrocellulose filters and incubated in 0.05 nM ^{125}I-IGF-I (16h, 5°C) with either native IGF-I (n-IGF-I) or b-IGF-I (using the biotinylation kit from Amersham) at 0.25-50 nM. Scatchard analysis of dot blot autoradiograms showed that the binding affinity of b-IGF-I and n-IGF-I were not significantly different (1-2 nM). To localize b-IGF-I with QAR, frozen sections from rat brain and bovine retina were incubated in 5 nM b-IGF-I (16h, 5°C), then incubated in ^{125}I-Streptavidin (2h, 23°C). Computer densitometry showed that b-IGF-I and ^{125}I-IGF-I co-localized to the same regions of the brain and retina. The results suggest that b-IGF-I is an effective probe for localizing IGF-I receptors. Furthermore, the results indicate that the dot blotting method is a valid approach for characterizing receptors on isolated cells. (Supported by NIH Grant NS 24809 and the V.A.).

394.5

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

Previously, we demonstrated that NG108-15 cells differentiated by treatment with 1.5% dimethyl sulfoxide (DMSO) and 0.5% fetal bovine serum for three to four days express both low and high affinity sites for angiotensin II (ANG II). The high affinity site is not expressed on undifferentiated cells, and its binding subunit has an approximate molecular weight of 74.5 ± 5.0 Kda by cross-linking analysis (Carrithers et al., FASEB J. 3(3):A732, 1989). In the present study, we further characterized the binding properties of both the low and high affinity sites. Using ^{125}I-ANG II, Scatchard analysis in the presence of 5 μM bestatin, a peptidase inhibitor, revealed a single low affinity site in undifferentiated cells (K_d ≃ 45 nM; B_{max} ≃ 320 fmol/mg protein) and a high and low affinity site in differentiated cells (K_{d1} ≃ 1.95 nM; B_{max1} ≃ 154 fmol/mg protein; K_{d2} ≃ 50.5 nM; B_{max2} ≃ 1676 fmol/mg protein). In competition studies, ANG III specifically displaced high affinity ANG II binding in differentiated cells (K_i ≃ 0.69 nM), but did not displace binding to the low affinity site in either differentiated or undifferentiated cells. We hypothesize that binding to the high affinity site in differentiated cells occurs through the carboxy-terminus of ANG II or ANG III and that it functions as a true receptor. It is further hypothesized that ANG II binds to the low affinity site through its N-terminus and that this site represents a bestatin-resistant aminopeptidase.

Supported by NSF BNS 17117 and NIH SITG GM07143.

394.6

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

Localization of brain AII receptors has previously been studied by quantitative autoradiography which has yielded valuable information on the distribution of receptors at the level of brain nuclei, but not at the cellular or subcellular levels. Therefore, we have developed a Bn-AII probe for use in the affinity cytochemical localization of AII receptors at the light and electron microscopic levels.

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

Rat brains were fixed with 2% paraformaldehyde-0.02 % picric acid in 0.1 M phosphate buffer, pH 7.5. Cryostat or vibratome sections were first incubated in an avidin-biotin "block" sequence followed by Bn-AII (1-100 nM) and avidin-peroxidase complex. The receptor-BnAII-Av-Po complex was visualized by incubation with DAB-0.5% nickel ammonium sulfate using the glucose oxidase system. Binding of Bn-AII was validated by addition of 1-10 uM AII in the cytochemical system and by characterization of binding using radioreceptor assays of fresh and fixed brain membrane preps. Staining distribution was limited to nuclei known to contain AII receptors. However, receptor localization was shown for the first time at high resolution in association with plasmalemma of neuronal and glial cells. From these studies, Bn-AII provides a valuable tool for the high resolution localization of AII receptors.

394.7

BINDING CHARACTERIZATIONOF A BIOTINYLATED ANGIOTENSIN II.
D.H. Harkness and M.S. Brownfield. University of Wisconsin School of Veterinary Medicine, Madison, WI 53706.

Binding characteristics of biotinyl angiotensin II (BnAII) were studied in saturation and competition studies. Crude membranes of brain, adrenal cortex, adrenal medulla, pituitary, liver, and kidney cortex were prepared from Sprague-Dawley rats. Iodinated BnAII, Sar1,Ile8-AII, and AII (2175 Ci/mmol) were incubated with membranes for 1 hr at 25°C in 50mM Tris HCl, pH 7.2, 150mM NaCl, 5mM EDTA, 2.5mM DTT, 0.2% BSA, 0.1mM PMSF. Bound ligand was separated from free by centrifugation.

Scatchard analysis showed the following K_Ds:

	AII	BnAII	Sar1,Ile8-AII
brain	.309nM	.125nM	.025nM
adrenal medullla	.204	.078	.095
adrenal cortex	.564	.325	.213
pituitary	.128	NB	.775
liver	.879	NB	.742
kidney cortex	.275	NB	1.230

NB=no binding up to 200 pM tracer concentration.

Preliminary studies using another buffer system with $MgCl_2$, EGTA, and 0.5mM DTT showed detectable BnAII binding in liver and kidney, with binding in the brain similiar to the previous buffer. IC50s for BnAII in brain membrane competition curves against ^{125}I-Sar1,Ile8-AII and ^{125}I-AII were 2.5nM and 5.9nM, respectively; however, IC50 for BnAII in liver membrane competition curves against ^{125}I-Sar1,Ile8-AII was 2.1uM.

These studies show that BnAII binds to AII receptors and suggest that BnAII may be recognizing CNS AII receptor subtypes not present in some other tissues.

394.8

PURIFICATION AND CHARACTERIZATION OF AN ANGIOTENSIN BINDING PROTEIN FROM BOVINE ADRENALS.
G.N. Swanson*, J.M. Hanesworth*, V.J. Cook, and J.W. Harding. Dept. of VCAPP, Washington State University, Pullman, WA 99164-6520.

An angiotensin binding protein from bovine adrenal cortical cell membranes was purified to homogeneity using a combination of centrifugation and chromatographic techniques. The purified protein which was still capable of specifically and reversibly binding angiotensins was identified as a single band on silver stained SDS-PAGE with an apparent molecular weight of 65,000. This glycoprotein exhibited an isoelectric point of 4.5 in its native form and 6.2 following treatment with neuraminidase. Preliminary data suggests that this protein may preferentially bind angiotensin III as opposed to angiotensin II.

394.9

FLUORO-GOLD DOES NOT ALTER ANGIOTENSIN II BINDING IN THE DORSOMEDIAL MEDULLA OF THE RAT. D.B. Averill, D.I. Diz, and S.H. Andreatta-Van Leyen*. Research Institute, Cleveland Clinic Foundation, Cleveland, OH 44195-5070.

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

	Intact	FG	NGX	VGX
% ipsi/contra	98	91±5	53±3	86±5
n	1	5	2	2

We conclude that after 2 week survival FG does not alter substantially AII binding in the dorsomedial medulla. The modest reduction in binding on the side of injection may be attributable to damage of fibers associated with injection of the tracer. These results suggest that FG may be used in combination with receptor autoradiography to determine the efferent projection of neurons whose binding sites are being studied. This work supported by NIH grants HL-6835, HL-36988 and HL-38535.

394.10

AUTORADIOGRAPHIC LOCALIZATION OF ANGIOTENSIN II RECEPTORS IN BRAIN AND PITUITARY OF DOCA-SALT HYPERTENSIVE RATS. D. P. Healy and N. Zhang*. Department of Pharmacology, Mount Sinai School of Medicine of the City University of New York, New York, NY 10029.

The activity of the brain angiotensin system (BAS) has been shown to be altered in several genetic hypertensive models and in response to changes in dietary sodium and to mineralocorticoid administration. In this report we examine the effects of deoxycorticosterone acetate-salt (DOCA-salt) induced hypertension on the expression of the brain angiotensin II (Ang II) receptor. Male Sprague-Dawley rats were uninephrectomized and assigned to one of four groups; either sham or DOCA-containing pellets on tap or salt water. After three weeks blood pressures were significantly elevated in the DOCA-salt group only. Animals were then processed for quantitative autoradiography using ^{125}I-sar^{1},ile^{8}-Ang II (^{125}I-SI-Ang II). There was a significant increase in the affinity and B_{max} of ^{125}I-SI-Ang II binding in the solitary-vagal area (SVA) from the DOCA-salt rats and an increased B_{max} in the DOCA rats. The density of ^{125}I-SI-Ang II binding was elevated in DOCA-salt rats in a variety of brain areas, including the circumventricular organs, the supraoptic nucleus and the paraventricular nucleus. In contrast, there was no significant change in ^{125}I-SI-Ang II binding in the anterior pituitary in any treatment group. These results suggest that the BAS may be involved in the expression of DOCA-salt hypertension. (Supported by USPH HL 42585.)

394.11

MODULATION OF ANGIOTENSIN II-INDUCED CYCLIC GMP PRODUCTION IN MURINE NEUROBLASTOMA N1E-115 CELLS BY PERTUSSIS TOXIN. X. Ye and S.J. Fluharty (Spon: M.A. Waraczynski) Depts. of Animal Biology and Biochemistry and Biophysics, and Institute of Neurological Sciences, Univ. of Pennsylvania, Phila., PA 19104.

In the murine neuroblastoma N1E-115 cell line angiotensin II (AngII) stimulated the rapid production of cyclic GMP (cGMP). cGMP production was maximal at 15 sec and continued agonist exposure resulted in diminished cGMP levels. Ang II stimulation was also dose-related with an apparent ED_{50} of 30 nM, and was maximal at 1 uM. Several AngII related agonists also increased cGMP levels and the rank order potency was AngIII > Ang II > Sarc^{1}-AngII > Angl. Sarc^{1},Ile^{8}-AngII, a high-affinity antagonist, completely blocked the stimualtion elicited by AngII. Finally, incubation of intact cells for 24 hr with pertussis toxin (100 ng/ml) resulted in an inhibition of subsequent pertussis toxin catalyzed incorporation of [^{32}P]ADP-ribose into a membrane protein of M_r 41kD. Pertussis toxin treatment also significantly attenuated AngII- and AngIII-induced cGMP production but did not alter the proportion of high affinity AngII receptors, or their regulation by guanine nucleotides. Collectively, these results suggest that cGMP may be an important intracellular mediator of Ang II actions within neuronal cells, and that pertussis toxin may modulate this transduction process through a mechanism independent of a change in agonist receptor affinity. Supported by NS 23986 and MH 43787.

394.12

A NOVEL PEPTIDE FUNCTIONS AS A BOMBESIN RECEPTOR AGONIST. M. Knight, T.R. Burke,Jr.*, S. Mahmoud* and T.W. Moody. Peptide Technologies Corp. Washington, DC 20017-1004 and Dept. Biochemistry, George Washington Univ. Sch. Medicine, Washington, DC 20037.

Bombesin (BN), a tetradecapeptide, is a neuromodulator in the CNS. The C-terminal octapeptide sequence of BN or gastrin releasing peptide is essential for binding to cell surface receptors, stimulating phosphoinositol turnover and elevation of Ca^{2+} cytosolic levels in rat brain slices and in the human glioblastoma cell line, U-118. Also BN stimulates the growth of Swiss 3T3 and human small cell lung cancer (SCLC) cells. Many linear BN analogs have been previously identified as BN receptor agonists or antagonists. Here a novel cyclic analog, cyclo[N-Ac,D-Lys^{5},D-Ala^{11},Met^{14}]BN(4-14) or cyclo mini BN has been synthesized. It inhibits the specific binding of [^{125}Tyr^{4}]BN with IC_{50} values of 0.5 uM in U-118 cells, 1 uM in rat brain and SCLC cells, and 2 uM in Swiss 3T3 cells. Also cyclo mini BN elevated cytosolic Ca^{2+} in SCLC and U-118 cells with ED_{50} values of 0.5 and 1 uM respectively. Additionally, the analog stimulated ^{3}H thymidine uptake in Swiss 3T3 cells with an ED_{50} of 0.3 uM, thus stimulating growth. Therefore this compound functions as a BN receptor agonist. The conformationally stabilized structure of cyclo mini BN may render it highly resistant to degradation and hence useful for in vivo studies. Grants SBIR NIH CA-44399 and NSF BNS-8815133

394.13

IDENTIFICATION OF HIGH AFFINITY ^{125}I-GASTRIN RELEASING PEPTIDE BINDING SITES ON RAT FOREBRAIN MEMBRANES. P.J. MONROE AND S.L. PEDROTTI. NOVA PHARMACEUTICAL CORPORATION, BALTIMORE, MD 21224.

In vivo and in vitro pharmacological and biochemical studies suggest that bombesin (BN) and gastrin releasing peptide (GRP) may act as neurotransmitters or neuromodulators in the central nervous system (CNS). In the present study, specific high affinity CNS sites for the putative mammalian analog of BN, ^{125}I-GRP, were characterized and compared to those previously reported for ^{125}I-tyr^{4}-BN (Moody, et al., Proc. Nat. Acad. Sci. 75, 1978).

Rat forebrain membranes were incubated with ^{125}I-GRP for 30 min. at 25°C in the presence of competing drug or buffer, then rapidly filtered under negative pressure onto Whatman GF/C filters. Specific ^{125}I-GRP binding, defined as that remaining in the presence of 1 uM BN, was routinely 66-75% of total radioactivity trapped onto the filters.

Analysis of saturation data indicate the existence of a single high affinity site (Kd = 0.23 nM; Bmax = 1.5 fmoles/mg tissue) comparable to that which has been reported for ^{125}I-tyr^{4}-BN. In addition, the rank order and selectivity of the ability of several related and unrelated peptides to compete for forebrain sites labelled by ^{125}I-GRP agree with that which has been previously reported for sites labelled by ^{125}I-tyr^{4}-BN. Thus, due to its mammalian origin, similar binding selectivity, and greater binding specificity, ^{125}I-GRP appears to be the preferred receptor probe for studying the sites mediating the action of BN in the mammalian CNS.

394.14

ONTOGENY OF BOMBESIN RECEPTORS IN THE RAT CNS. T.W. Moody, R. Getz*, C. Merchant* and Z. Merali. Dept. Biochem. & Molecular Biology, The George Washington Univ. Med. Ctr. Washington, D.C. 20037 and Dept. Psychology, Univ. Ottawa, Ottawa, Ontario, KIN 9A9.

Bombesin (BN), a potent grooming and satiety agent, represents one class of neuropeptides biologically active in the CNS. Previously, we found that BN-like peptides increase dramatically during the third postnatal week (Gillati, M.G. and Moody, T.W., Dev. Brain Res. 15:286, 1984). Here we investigated the ontogeny of BN receptors using in vitro autoradiographic techniques. Fresh frozen sections of rat brain were prepared and the distribution of (^{125}I-Tyr^{4})BN binding sites determined as described previously (Zarbin M.A. et al. J. Neurosci. 5:429, 1985). Autoradiagraphic grains were present in E18 and maximal in P1 animals. High densities of BN receptors were present in the olfactory bulb and tubercle, n. accumbens, hypothalamus, hippocampus, inferior colliculus and hindbrain. The density of BN receptors was increased in the P1 animals. The grain density declined during the first postnatal week especially in the inferior colliculus and hindbrain and in P7 animals the BN receptor density closely resembeled that of the adult. Because BN receptors undergo a major redistributions during development, they may facilitate the differentiation of the brain. Supported by NSF grant BNS-8815133.

394.15

CHARACTERIZATION OF RODENT PITUITARY AND CELL LINE GNRH RECEPTORS EXPRESSED IN RNA-INJECTED XENOPUS OOCYTES. S.C. Sealfon*, B. Gillo*, S. Mundamattom*, P. Mellon*, J. Windle*, E. Landau and J. L. Roberts (SPON: G. Cohen). Fishberg Center in Neurobiology, Neurology, Psychiatry, Mount Sinai School of Medicine, New York, N.Y. 10029 and Salk Institute, La Jolla, CA.

GNRH receptors were expressed in *Xenopus* oocytes micro-injected with RNA isolated from rat pituitary and from the αT3 cell line. 3-4 days after RNA injection, cells were recorded by voltage clamp. On exposure to 10^{-7} M GNRH an inward current of 532±87 nA (n=20) was obtained in pituitary RNA injected cells and 1163±526 nA (n=3) in αT3 RNA injected oocytes. The threshold for obtaining a response after pituitary RNA injection was $3X10^{-9}$ M GNRH. Equimolar concentration of a GNRH antagonist reduced the reponse to GNRH by greater than 90%, while the response to TRH was unchanged. Injection of sucrose gradient RNA fractions showed the receptor mRNA(s) has a sedimentation factor larger than 28S. Studying the molecular mechanism, the response to GNRH was not changed in calcium-free medium. In cells injected with a mixture of brain and pituitary RNA, the response to GNRH was eliminated by intracellular injection of EGTA whereas the response to GABA was not affected. Ramps studies revealed a reversal potential of the GNRH generated current of -22.5 ±1.0 mV (n=7) and -25.6±3.3 mV(n=3) in pituitary and cell line RNA injected oocytes respectively, consistent with the chloride reversal potential. The GNRH response is mimicked by intracellular injection of IP3. Normal rat pituitary and αT3 cell line RNA lead to the expression of functional GNRH receptors in Xenopus oocytes. The response is dependent on intracellular but not extracellular calcium and represents a calcium-dependent chloride conductance, suggesting signal transduction by inositol phosphate metabolism.

394.16

ASSESSMENT OF SOMATOSTATIN-ACETYLCHOLINE INTERACTIONS IN THE RAT CEREBRAL CORTEX. L.L. Cook, G.A. Faux,* D.L. Knight, G. Bissette and C.B. Nemeroff. Depts. Psychiat. & Pharmacol., Duke Univ. Med. Ctr., Durham, NC 27710.

Somatostatin (SRIF) fulfills many of the requisite neurotransmitter criteria and modulates certain neurophysiological and neurochemical actions of acetylcholine. These studies were designed to evaluate a possible localization of SRIF receptors on cholinergic afferents in the rat frontal cortex (FC), and a possible SRIF modulation of carbachol-stimulated phosphoinositol (PI) hydrolysis in FC slices. Nucleus basalis lesions were performed using 2 bilateral ibotenic acid infusions (22 and 8 days prior to sacrifice; 5 μg/0.5 μl/site, or 0.5 μl vehicle/site). Choline acetyltransferase activity was reduced by 41% in the FC and by 46% in the parietal cortex. Scatchard analysis of [^{125}I-Tyr-11]-SRIF binding in FC synaptosomal membranes showed that the mean Km and Bmax values in lesioned and control rats were not different ($p > 0.22$, ANOVA). In addition, the stable SRIF analog, SMS 201-995 (Sandoz), did not alter carbachol-stimulated PI hydrolysis in FC slices in concentration studies with this peptide or with carbachol. SRIF receptors are apparently not localized on cholinergic afferents in the FC, and SRIF apparently does not modulate the second-messenger activity associated with post-synaptic (M1) muscarinic receptors. (Supported by NIMH MH-40524 and NRSA ES07031).

394.17

CYCLIC ANALOGUES OF SOMATOSTATIN DISTINGUISH PHARMACOLOGICALLY AND FUNCTIONALLY DISTINCT SUBTYPES OF SOMATOSTATIN RECEPTORS IN BRAIN AND PITUITARY. K. Raynor and T. Reisine. Dept. of Pharmacology, Univ. of PA, Phila., PA 19104.

Somatostatin (SRIF) is a tetradecapeptide found widely distributed throughout the CNS where it serves as a neurotransmitter. SRIF induces its cellular effects in brain, including inhibition of adenylyl cyclase activity and modulation of ionic currents, through specific membrane receptors. The different physiological actions of SRIF may be mediated by distinct receptor subtypes. The lack of availability of subtype specific compounds has hindered the definition and functional characterization of SRIF receptor subtypes in the brain and periphery. Employing the SRIF analogues CGP 23996 and MK 678, we have demonstrated the existence of pharmacologically and functionally distinct SRIF receptor subtypes in the rat brain. Within the brain, [125I] MK 678 binding is of high affinity and saturable. It is also specific for SRIF receptors, being displaced by low nM concentrations of SRIF and its analogues, but not by other unrelated peptides. [125I] CGP 23996 labels a SRIF receptor which is poorly recognized by MK 678 and its structurally similar analogues, even at concentrations as high as 10 uM. These and other data suggest that [125I] CGP 23996 and [125I] MK 678 label distinct SRIF receptor populations in the CNS. In contrast, [125I CGP 23996 and [125I] MK 678 appear to label a single receptor population in pituitary. Both MK 678 and SRIF inhibit forskolin-stimulated adenylyl cyclase activity in pituitary. While SRIF also inhibits adenylyl cyclase activity in the corpus striatum, a region of brain with a high density of SRIF receptors, MK 678 has no effect. These findings suggest that in some tissues, subtypes of SRIF receptors may be coupled to different cellular effector systems. Subtype selective analogues of SRIF may therefore be useful in identifying the receptor subtypes involved in mediating the diverse biological actions of somatostatin in the brain. Supported by grants from the NIH (GM34781) and ONR (14-88-K-48).

394.18

BIOCHEMICAL PROPERTIES OF THE SOLUBILIZED BRAIN SOMATOSTATIN (SRIF) RECEPTOR. H-T. He*, S. Rens-Domiano, S. Borislow*, and T. Reisine. Dept. of Pharmacology, Univ. of PA 19104.

SRIF is a neurotransmitter in the CNS whose physiological actions are mediated by membrane-bound receptors. To gain insight into the molecular mechanisms by which SRIF exerts its actions in the CNS, we have investigated the biochemical properties of the solubilized brain SRIF receptor. Brain SRIF receptors were solubilized with CHAPS and detected with the high affinity SRIF agonist [125I] MK 678. The equilibrium binding of [125I] MK 678 to the solubilized SRIF receptor was saturable and selectively blocked by SRIF agonists. The solubilized SRIF receptor appears to be tightly associated with GTP binding (G) proteins since all specific [125I] MK 678 binding to the solubilized receptor was inhibited by GTPgS. Furthermore, antibodies directed against fragments of the alpha subunit of G proteins partially precipitated the solubilized SRIF receptor and pertussis toxin treatment of AtT-20 cells, a tumor cell line with high expression of SRIF receptors, caused the uncoupling of G proteins from the SRIF receptor and abolished [125I] MK 678 labeling of the solubilized SRIF receptor. The solubilized SRIF receptor appears to be a glycoprotein since the receptor could be eluted from a WGA column with the sugar TACT. Studies are in progress to determine whether subtypes of solubilized SRIF receptor couple with different G proteins and have different physical properties. Supported by NIH grant GM 34781 and ONR (14-88-K-0048).

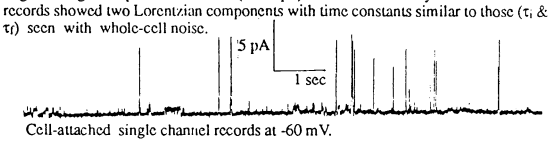
POTASSIUM CHANNELS IV

395.1

Three Kinetic Components to M-Currents in Bullfrog Sympathetic Neurons. W. Gruner*, N.V. Marrion* and P.R. Adams. Howard Hughes Medical Institute, SUNY at Stony Brook, Stony Brook, NY 11790, USA.

We have used noise analysis and time-course determination of whole-cell macroscopic currents, together with noise analysis of single-channel recordings and unitary current analysis to examine the kinetics of M-current in dissociated bullfrog sympathetic neurons. Whole-cell deactivation currents, evoked by hyperpolarizing voltage commands from holding potentials of ~-30 mV, in both 2.5 mM and 110 mM K^+ solutions, showed two muscarine-sensitive components; a slowly decaying portion (τ_s) in the 100's of msecs (c.f. Adams et al., *J. Physiol.*, 330:537-572) and a faster declining phase (τ_i). τ_i varied between 70 and 10 msecs over a voltage range of -30 to -90 mV, showing a slight voltage-dependence (~e-fold in 40 mV) at hyperpolarized potentials, while τ_s showed no obvious voltage-dependence. Fluctuation analysis of whole-cell currents revealed two Lorenztian components, one of which was similar to the macroscopic current τ_i, in both time course and voltage-dependence. The other, a much faster component, had a time constant (τ_f) between 3 and 5 msecs and showed no obvious voltage dependence. Both of these components were sensitive to muscarine (c.f. Neher et al, *Febs Letts.*,240:88-94, 1988, Marsh & Owen, *J. Physiol.*, 410:31P, 1988).

Single channel recordings of cell-attached patches with 110 mM K^+ in the patch pipette, have revealed two K^+ channels; one (~15 pS) which exhibited a voltage-dependence similar to that observed with whole-cell noise and current analysis, and a larger voltage-independent channel (~105 pS). Fluctuation analysis of the channel records showed two Lorentzian components with time constants similar to those (τ_i & τ_f) seen with whole-cell noise.



Cell-attached single channel records at -60 mV.

395.2

M-CURRENT NOISE IN RAT SYMPATHETIC NEURONES. **D.G. Owen[§], S.J. Marsh* and D.A. Brown. Dept. Pharmacology, University College London, London WC1E 6BT, U.K.**

Muscarine blocks the non-inactivating voltage-activated K^+ current, I_M, in rat sympathetic cervical (SCG) neurones (see Brown, 1988). In order to estimate the parameters of single ion channels underlying whole cell M-currents, we applied the technique of noise analysis to muscarine-sensitive currents. *Whole Cell* recordings were made from cultured (ca. 7 days) adult SCG neurones using a standard extracellular solution and patch pipette solution containing *inter alia* 100nM free Ca^{2+} and 1mM ATP at pH 6.8. Currents recorded under voltage clamp before, during and after the application of muscarine were stored on VCR, and subsequently bandpassed (0.05-50Hz) before acquisition by a PDP-11/23 via CED 502 interface. Spectral analysis was carried out using *NOIS* (D. Colquhoun). Data acquired at -30mV was found to lie within the low probability limit and power spectra at this potential were always fit by two Lorentzian Functions (*eg.* f_c^1 = 1.0Hz, $S(\phi)$ =1.5pA^2; f_c^2 = 32Hz, $S(\phi)$ = 0.02pA^2). Results were consistent with a single M-channel of about 1.6pS, having at least 3 states. The time constant corresponding to f_c^1 is 159ms which is similar to that of *macroscopic* I_M deactivation tails at the same membrane potential (-30mV).

Brown, D.A. (1988) TINS, 11, 294-299.

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395.3

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

Acetylcholine (ACh) causes an increase in the probability of opening inwardly rectifying K^+ channels on heart nodal cells. In this study we examined the effect of ACh on the K^+ inward rectifier of skeletal muscle. Muscle cultures were prepared from Xenopus myotomal muscle and macroscopic whole cell currents were recorded using a List patch clamp. The nicotinic ACh receptors and Na^+ currents were blocked with bungarotoxin (10^{-5} g/ml) and tetrodotoxin (10^{-6} g/ml), respectively. The external recording solution contained (mM) 140 NaCl, 5 KCl, 1 $CaCl_2$, 1.2 $MgCl_2$, 10 HEPES (pH 7.4) and the solution in the patch electrode contained (mM) 140 KCl, 1 EDTA, 5 $MgCl_2$, 10 HEPES (pH 7.4). Hyperpolarizing step potentials from resting membrane potential resulted in an inward current that was similar to the K^+ inward rectifier on adult skeletal muscle. Application of ACh (0.1-10 mM) to the bath did not alter the amplitude, time course or activation of inward current on these muscle cells. In contrast to the effects on heart nodal cells, ACh does not alter the properties of the classic inward rectifier. (supported by MRC, Canada)

395.4

MUSCARINIC AND NORADRENERGIC EFFECTS ON VOLTAGE DEPENDENT POTASSIUM CURRENTS IN CAT BLADDER PARASYMPATHETIC NEURONES.

Eva Vlckova* and Patricia Shinnick-Gallagher. Department of Pharmacology and Toxicology, University of Texas Medical Branch, Galveston, TX 77550

In cat bladder neurones, acetylcholine and noradrenaline have both excitatory and inhibitory effects. In this study we analyzed the effects of these neurotransmitters on voltage dependent K^+ currents using single-electrode voltage clamp. Several types of voltage dependent K^+ currents were observed in these neurones. These currents possess a similar voltage dependence and pharmacology as those described previously in other types of central and peripheral neurones and include: a M-current suppressed by Ba^{2+} (0.3-1.0 mM) and muscarine (10-20 uM); an A-current suppressed by 4-aminopyridine (1.0 mM); a Ca^{2+}-dependent K^+ current sensitive to Co^{2+} and Cd^{2+}, suppressed by apamin (1.0 uM) and not affected by TEA (2.0-4.0 mM), a Q-current blocked by extracellular Cs^+ (3.0 mM) and an anomalous rectifier current which is blocked by Ba^{2+} and Cs^+ and seems to be Ca^{2+}-dependent. Both muscarine (3-10 uM) and noradrenaline (10 uM) induced a net outward current and enhanced anomalous rectification. Muscarine (10-20 uM) suppressed I_M and $I_{K(Ca)}$, while noradrenaline appeared to have no effect on either of these currents. (Supported by NS16228)

395.5

CYCLIC AMP DEPENDENT CATION CURRENT IN DISSOCIATED BULLFROG SYMPATHETIC AND PRIMARY AFFERENT NEURONS. T. Tokimasa, H. Hasuo*, T. Nishimura*, M. Tsurusaki*, M. Shiraishi* and T. Akasu*. Dept. of Physiology, Kurume Univ. Sch. of Med., Kurume 830, Japan.

A hyperpolarization-activated sodium/potassium current (gH) was recorded from cultured bullfrog sympathetic neurons in the whole-cell configuration. gH activated between -130 and -60 mV (50 % activation between -90 and -95 mV). A hydrolyzable form of ATP in a pipette solution was necessary for gH. Bath application of ATP (1 μM) was without effects on gH while the drug selectively blocked the M-current (gM). The maximum conductance of gH was increased by intracellular "loading" of cyclic AMP (3-10 μM), or bath application of forskolin (0.1-10 μM), membrane permeable cyclic AMP analogues (1 mM) and IBMX (0.1-1 mM). Open/close kinetics of gH showed a depolarizing shift when it was facilitated by intracellular cyclic AMP. gM but not gH was depressed by C-kinase activator phorbol 12-myristate 13-acetate (3 μM). The amplitude of gH was approximately halved by protein kinase inhibitor H-8 (3 μM). Essentially the same results were obtained from cultured dorsal root ganglion cells. It is concluded that a voltage-gated cation current is also controlled by basal activity of cyclic AMP presumably via protein kinase-A. Supported by the Naito Foundation (#87-128).

395.6

***PANDINUS Imperator* VENOM BLOCKS VOLTAGE-ACTIVATED K-CURRENTS OF FROG SKELETAL MUSCLE.** D.L.Eng* and P.A.Pappone (SPON;A.J.Gabor). Dept. Animal Physiol., Univ. of Calif., Davis, CA 95616.

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

Bullfrogs were decapitated and pithed, and the semitendinosus muscle was dissected and placed in frog Ringers. Single muscle fibers were mounted in the Hille-Campbell vaseline gap chamber. The fiber ends were cut in 80mM K-EGTA to maintain internal calcium concentrations at low levels to prevent activation of contraction and calcium-activated K currents. Voltage clamp records were obtained in either 0.1% BSA frog Ringer, pH 7.4 to test for $I_{K,DR}$ or in a 0.1% BSA K-methylsulfonic acid, pH 7.4 solution to test $I_{K,IR}$ Holding potentials were -80mV in Ringers, and 0mV in 120mM KMSA. Currents were measured in the voltage clamp mode in response to both steps and ramps of command voltage. Untreated Pandinus venom (200 ug/ml) blocked 50% of $I_{K,DR}$ but had no significant effect on $I_{K,IR}$. *Pandinus*, neither heat-inactivated (200ug/ml) nor dialysed (500ug/ml) venom had any significant effect on $I_{K,DR}$. 200 uM zinc, a cofactor of *Pandinus* venom, had a similar but smaller effect than whole venom on $I_{K,DR}$. CTX (200nM) and apamin (500nM), two blockers of different Ca-activated potassium channels, had no significant effects on frog skeletal muscle K currents, $I_{K,DR}$ or $I_{K,IR}$. Previously it was believed that *Pandinus* venom did not block $I_{K,DR}$ of frog skeletal muscle, and that all the effects seen were due to zinc contained in the venom. Here we show that whole *Pandinus imperator* venom can have effects on the $I_{K,DR}$ of frog skeletal muscle. (NIH AR-34766, NS07300)

395.7

CROMAKALIM ACTIVATES A TETRAETHYLAMMONIUM-SENSITIVE VOLTAGE-DEPENDENT K+ CURRENT IN CULTURED RAT HIPPOCAMPAL NEURONS. D.M. Politi*, S. Suzuki* and M. A. Rogawski. Medical Neurology Branch, NINDS, NIH, Bethesda, MD 20892.

The whole cell voltage-clamp recording technique was used to study the effects of the benzopyran antihypertensive agent cromakalim (BRL 34915) on voltage-dependent outward currents in cultured embryonic rat hippocampal neurons. Extracellular perfusion with cromakalim (10-500 μM) produced a dose-dependent increase in the sustained (minimally inactivating) voltage-dependent outward current (I_K) without altering the transient outward current (I_A) or producing a change in the resting current at -60 mV. One-half maximal facilitation of I_K occurred at 40 μM and maximal facilitation (59%) at 100 μM. The increase in outward current occurred at all potentials where hippocampal neurons exhibited outward rectification (> -30 mV). The effect of cromakalim was slow to develop (requiring as long as 5-15 min to achieve maximal effect) and persisted for at least 10-15 min after cessation of drug superfusion.The outward current activated by cromakalim was blocked by 20 mM tetraethylammonium (TEA). However, despite the failure of cromakalim to have an observable effect in the presence of TEA, the outward current rapidly recovered to a potentiated level when the drugs were both withdrawn.These results indicate that cromakalim can enhance the activity of TEA-sensitive, voltage-dependent K+ channels in mammalian CNS neurons. Moreover, cromakalim appears to be capable of interacting with its acceptor even when current flow is blocked by TEA.

395.8

APAMIN-SENSITIVE CONDUCTANCE IN LOCUS COERULEUS NEURONS. S.S. Osmanović, S.A. Shefner and B.B. Beleslin*, Dept. Patho-Physiol. Med. Fac. Belgrade, Yugoslavia and Dept. Physiol. Biophys., Univ. Illinois. Col. Med., Chicago, IL.

Apamin, a bee venom neurotoxin, has been shown to selectively block one class of Ca^{2+}-activated K channels, $g_{K(Ca)}$. Intracellular recordings were made from rat locus coeruleus (LC) neurons in completely submerged brain slices under current- and voltage-clamp conditions. Bath superfusion of 100-500 nM apamin affected both the single spike after-hyperpolarization (AHP) and the post-stimulus hyperpolarization (PSH) which follows a train of action potentials. Apamin reduced the magnitude of the late part of the single spike AHP with no change in its peak amplitude. In most LC neurons, PSH elicited by a train of action potentials consisted of two distinct phases which could be fitted by a biexponential function. The initial, fast phase had a decay constant one order of magnitude smaller than that of the late, slow phase. Both phases were abolished in low Ca^{2+} medium and by Ca-channel blockers, which suggests that they are mediated by an activation of $g_{K(Ca)}$. Apamin abolished the initial, fast component of PSH and augmented the late component. Apamin also increased the number of spikes evoked by a long depolarizing pulse. These data suggest that apamin-sensitive $g_{K(Ca)}$ underlies the late part of the single spike AHP and a fast phase of PSH, and is involved in regulation of repetitive firing in LC neurons.

Grant support: PHS AA-5846 (S.A.S.)

395.9

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

Whole-cell and excised outside/out patch clamp techniques were used to study the effects and mechanisms of tetraethylammonium (TEA) and quinine on Ca-activated K [K(Ca,V)], ATP-inhibited K [K(ATP)] channels and delayed rectifier current in HIT cells. HEPES-buffered (pH 7.2) solutions (5mM K, 120mM Na ± Cd 200μM outside; 140mM K, ±100μM Ca, ±10mM ATP inside) was used at 20-22 °C. TEA reversibly and rapidly reduced the open channel conductances of K(Ca,V) and K(ATP) and reduced delayed rectifier current. It was more effective on K(Ca,V) (Kd≤200μM, n=8) than on K(ATP) (Kd≥20mM, n=8) and delayed rectifier current (Kd≥200μM, n=3). TEA did not affect gating of K(Ca,V) and K(ATP) channels. Quinine decreased open channel conductance of K(Ca,V) (Kd≥500μM, n=6) and reduced delayed rectifier current (Kd≥600μM, n=3). On the other hand, quinine did not affect K(ATP) single channel conductance but did reduce the frequency of channel opening (Kd≤100μM, n=7).

Conclusion: Low doses (<mM) TEA provides relatively specific block of K(Ca,V) channels while low doses (<100μM) of quinine is relatively specific for K(ATP) channels. Quinine inhibits K(ATP) by interfering with channel opening while it inhibits K(Ca,V) channels by directly blocking K flux through open channels.

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

395.10

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

Although general anesthetic actions on free nerve endings are not thought to contribute greatly to anesthesia, few detailed studies are available to support this. The present study investigated the effects of halothane and isoflurane on A and C fiber nociceptors using a new in vitro preparation from rabbit cornea. The anesthetics produced concentration and time dependent changes in action potential discharge activity. Increased discharge frequency was produced by low concentrations (0.5-0.8 x MAC), burst discharges occurred at 1-2 x MAC and depression of discharge was observed at concentrations > 2.5 x MAC. The potassium channel blocker 4-AP (0.25 mM) produced a similar profile of excitation and burst firing; TEA, Ba, Gd and Quinine did not. The anesthetics and 4-AP also increased spike width and depressed the after potential in a concentration dependent manner, however 4-AP produced an increase in spike amplitude while the anesthetics depressed amplitude by 20% at 1 MAC. The results indicate that clinically relevant concentrations of volatile anesthetics excite free nerve endings by depressing a potassium current. In addition, the anesthetics produce a decrease in spike amplitude. These effects on peripheral fibers would be expected to antagonize the central depressant actions of the anesthetics by increasing excitatory afferent input to the CNS. Supported by the American Cancer Society and a Parker B. Francis Investigatorship in Anesthesiology.

POTASSIUM CHANNELS: MODULATION AND REGULATION

396.1

MODULATION OF FIRING BEHAVIOR AND AFTERHYPERPOLARIZATIONS IN SENSORIMOTOR CORTEX BY SEROTONIN (5HT). R.C. Foehring. Dept. of Anatomy and Neurobiology, Univ. of Tenn., Memphis, TN, 38163.

Neocortical neurons were studied *in vitro* in brain slices from pericruciate cortex of cats and the sensorimotor region of rat cortex. Neuronal firing behavior and responses to 5HT were similar for neurons from the two species. Bath application of 5HT (10-100 μM) resulted in (i) an initial hyperpolarization (brief and variably present), (ii) a prolonged depolarization which was usually associated with an increase in input resistance, and (iii) a reduction in the slow afterhyperpolarization seen following sustained repetitive firing. In the presence of 5HT, cortical neurons fired faster in response to a given current injection, exhibited less spike-frequency adaptation, and showed less habituation to repeated 1s suprathreshold stimuli. Single electrode voltage clamp suggests that the slow afterhyperpolarization reduction was due to a reduction in the slower Ca^{2+}-dependent K^+ current. 5HT also appears to reduce the duration of the medium afterhyperpolarization seen following a single action potential. Several agonists and antagonists were studied to deduce the 5HT receptor subtypes involved in the above responses.

396.2

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

Whole cell currents from human cortical neurons were assayed for changes with growth conditions. Neurons isolated from a case of childhood megalencephaly were passed by trypsinization, plated for 2 days then differentiated with nerve growth factor, isomethylbutylxanthine (IBMX) and dibutyryl cyclicAMP (dibut cAMP) for 2 days. Cells grew long branched processes, expressed neurofilament protein, and neuron specific enolase. Whole cell currents were measured using patch pipettes containing in mM: 5 MgATP, 1 KEGTA, 40 KCl, and 110 Kaspartate buffered to pH 7.4 with Hepes Tris. The bath contained 140 NaCl, 5 KCl, 1 CaCl, 1 MgCl, 20 glucose and NaHepes pH 7.4. Under these conditions the major current was a voltage-dependent outward current which did not inactivate during 250 ms depolarizing pulses and increased 2x when the holding potential (HP) was shifted from -40 mV to -100 mV. At a HP of -100 mV and depolarization to +50 mV the outward current was 800 pA. With 0.5 mM IBMX and 0.5 mM dibut cAMP in the bath, a new outward current appeared which showed voltage-dependent inactivation. With HP -100 mV and depolarization to +50 mV this outward current was 120 pA and inactivated within 50 ms. We conclude that rapidly inactivating currents are by modulated increased intracellular cAMP.

396.3

CALCITONIN GENE-RELATED PEPTIDE (CGRP) DECREASES POTASSIUM CURRENTS IN RAT NEOCORTICAL NEURONES.
C. ZONA, E. PALMA*, F. EUSEBI*. Dipt. Med. Sper. Sci. Biochem. Univ. Roma "Tor Vergata" - Dipt. Med. Sper. Univ. L'Aquila, Italy.

CGRP, a 37 amino acid peptide, identified in spinal cord of vertebrate and in motor nerve endings of mammalian neuromuscolar junction, is known to stimulate the biosynthesis of cyclic AMP, the physiological activator of protein kinase A. In order to investigate whether CGRP may regulate the voltage-dependent channels function, experiments were performed on neocortical neurones from 15 - day rat embryos grown in dissociated cell culture for 1 - 6 weeks. Outward currents evoked by depolarization commands from - 80 mV holding potential exhibited a reversible decrease in size within 1-3 min CGRP application (500 nM - 1 μM). The effect of CGRP on the outward currents was more potent in decreasing the K+ fast transient current than the delayed rectifier K+ current and was mimicked by the stimulator of the adenylate cyclase forskolin (20 μM). It has been supported that CGRP may affect voltage-dependent K+ channels through the stimulation of protein kinase A.
This work is supported by a grant from Fidia.

396.4

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

The neuropeptide galanin initiates a slowly developing and longlasting hyperpolarization of neurons in the mudpuppy (Necturus maculosus) cardiac ganglion by activating a membrane K^+ conductance. (Konopka et. al, J. Physiol. 410: 107, 1989). We demonstrate in the present study that following substitution of Mn^{2+} or Mg^{2+} for extracellular Ca^{2+}, the amplitude of the galanin induced hyperpolarization is decreased reversibly. Also, following the addition of 0.1mM Cd^{2+} to the 3.6mM Ca^{2+} containing solution, the galanin-induced hyperpolarization decreased progressively. The galanin-induced hyperpolarizations were not reduced in the presence of 5-10 mM TEA, but were significantly decreased (40-100%) during exposure to 25uM apamin. The spike HAP also was decreased in many cells by 25uM apamin. These results indicate that galanin initiates hyperpolarization by activating a Ca^{2+} dependent K^+ conductance. Further, we suggest that the channels activated are similar to the Ca^{2+}-dependent K^+ channels mediating the spike HAP.
Supported by PHS Grants NS 23978 and NS 25973.

396.5

MODULATION OF MEMBRANE CURRENTS BY FMRFAMIDE AND MYOMODULIN IN TAIL SENSORY NEURONS OF *APLYSIA*. S.D. Critz, D.A. Baxter and J.H. Byrne. Department of Neurobiology and Anatomy, University of Texas Medical School, Houston, TX 77225.

We have shown that the neuropeptides FMRFamide and myomodulin can reverse the increases in neuronal excitability and spike duration induced by the application of serotonin to tail sensory neurons in *Aplysia* (Cleary et al., 1987). Therefore, we were interested in determining which membrane currents mediate these effects. Clusters of sensory neuron somata, isolated from pleural ganglia and maintained at 15° C in a static bath of ASW containing 200 μM TTX, were examined by conventional two-electrode voltage clamp techniques. Membrane currents were activated by 200 msec voltage clamp pulses from a 15 sec prepulse to -70 mV. Voltage clamp pulses were separated by 60 sec during which the cells were held at the resting membrane potential. Current responses were digitized and stored for later display and analysis.

The effects of FMRFamide were complex. At low voltage clamp potentials (-30 to -10 mV), bath application of FMRFamide (50-100 μM) resulted in an increase in the net outward current at the end of the pulse. Computer subtractions indicated that the outward current increased by FMRFamide was similar to the S-current. It was slowly activating, non-inactivating, relatively voltage-independent, and insensitive to TEA and 4-AP. At higher voltages (0 to 20 mV), FMRFamide caused a large decrease in the net outward membrane current at the end of the pulse. This component of the FMRFamide response was blocked by a low concentration of TEA (2 mM), which blocks $I_{K,Ca}$, and was unaffected by 4-AP (1.0 mM), which blocks I_A and $I_{K,V}$ but not $I_{K,Ca}$ (Baxter and Byrne, 1989), in these cells. These results indicated that an $I_{K,Ca}$ was reduced at these depolarized potentials. In contrast, myomodulin (10-50 μM) resulted in a TEA and 4-AP insensitive increase in the net outward current at the end of all voltage steps. Thus, both FMRFamide and myomodulin appear to increase the S-current, but FMRFamide appears to reduce $I_{K,Ca}$, as well. We are presently investigating whether other currents are modulated by these peptides.

396.6

GTP-BINDING PROTEINS MEDIATE DOPAMINE ACTIVATION OF A POTASSIUM CURRENT IN IDENTIFIED RAT LACTOTROPHS. L.C. Einhorn and G.S. Oxford. The Neurobiology Curriculum, University of North Carolina, Chapel Hill, NC 27599.

Dopamine (DA) is the major physiological regulator of prolactin (PRL) secretion from the anterior pituitary, exerting a tonic inhibitory control. Utilizing the reverse hemolytic plaque assay and whole cell patch clamp techniques, we investigated the actions of DA on the membrane potential and ionic conductances in primary rat lactotrophs as well as the signal transducing mechanisms which may be involved. Application of DA (5nM-5μM) or a D2 agonist (RU24213) evoked a 20mV hyperpolarization of the membrane potential which was blocked by D2 selective antagonists and associated with an increased K^+ conductance. Whole cell current responses in voltage clamp revealed a DA-activated current whose reversal potential was near the K^+ equilibrium potential and varied with changes in extracellular [K^+]. The DA-activated current exhibited a slight inward rectification. The response to DA was present when both extracellular and intracellular Ca^{++} levels were buffered to < 10^{-7}M or when the cell was dialyzed with 2mM cAMP. The current was insensitive to TEA (10mM), only partially blocked by 4AP (≥5mM), and almost completely inhibited by quinine (100μM). Pretreatment of cells with *Pertussis* toxin (PTX; 500ng/ml, 4-8hrs) or intracellular dialysis with GDPβs (100-500μM) abolished the DA induced membrane hyperpolarization, whereas GTPγS (50μM) dialysis mimicked the DA response. These findings suggest that the mechanism linking D2 receptors to inhibition of PRL secretion involves G-protein activation of a K^+ current which functionally decreases membrane excitability. Supported by NIH Grant NS18788.

396.7

MODULATION OF NEURONAL ION CHANNELS BY PROTEIN PHOSPHORYLATION AND DEPHOSPHORYLATION. S. Chung*, P. Reinhart*, B. Martin*, D.L. Brautigan*, and I.B. Levitan. Biochem. Dept., Brandeis Univ., Waltham, MA 02254 and Section of Biochem., Div. Biol. and Medicine, Brown Univ., Providence, RI 02912.

Rat brain plasma membrane vesicles contain ion channels which can be incorporated into lipid bilayers. We have observed a family of four calcium-dependent potassium channels, which share some properties and differ in others (Reinhart et al, **Neuron**, 2:1031, 1989). All of the channels are highly selective for potasssium over sodium (and anions), and are activated by calcium in the micromolar range. Among them are two kinetically distinct maxi-channels. They have similar single channel conductances (about 240pS), and both are sensitive to TEA. However charybdotoxin blocks only the faster gating channel, and they also differ in their calcium sensitivity, gating kinetics, and modulatability. The activity of the faster gating channel is increased by addition of the catalytic subunit of cAMP-dependent protein kinase to the bilayer system. In contrast the activity of the slower gating channel is *decreased* by the catalytic subunit. In both channels the catalytic subunit effects are reversed by the addition of the purified phosphoprotein phosphatase 2A. The slower gating channel is up-regulated by ATP, and this effect is not reversed by phosphatase 2A but is reversed by phosphoprotein phosphatase 1. Since the regulation by ATP occurs in the absence of exogenous kinase, it may involve the action of some endogenous protein kinase, not cAMP-dependent, which is present in the plasma membrane vesicles and which may accompany the channel when it inserts into the bilayer. Thus this channel may be the target for modulation, in opposite directions, by two distinct protein kinases.

396.8

MODULATION OF SINGLE CALCIUM- AND VOLTAGE-DEPENDENT K^+ CHANNELS FROM RAT BRAIN BY A- AND C-KINASES. J. Farley, Prog. in Neural Science, Indiana University, Bloomington, IN 47405.

I have assessed the effects of purified A- and C-kinases upon the activity of a family of voltage-, calcium-dependent, and CTX-inhibited K channels of relatively large conductance (Biophys. J., 1988, 53; 919), found in rat brain synaptosomal membrane vesicles, incorporated into bilayers on the tips of patch-electrodes.

Catalytic subunit of cAMP-PK (A-kinase) (nM) increased the open-time probability of large (>200 pS) "BK" channels, as well as that of two intermediate-sized channels (~75 and 125 pS, respectively). Both the rate of opening and open-time duration were affected, depending upon the channel type. A fourth (~50 pS) K^+ channel was unaffected by A-kinase. Exposure of channels to a heterogeneous mix of purified PKC isozymes and activators (Ca^{2+} and diG) failed to consistently affect the BK channel, but produced profound decreases in open-time of the two intermediate-sized channels. The open-time of the fourth (~50 pS) channel was increased. All enzyme effects were ATP-dependent, and were not observed if heat-inactivated PKs were used. In some experiments, however, exposure of membranes to C-kinase activators alone (no exogenous enzyme) also produced closures of the intermediate-sized channels, suggesting that endogenous enzyme may have remained associated with these membranes.

396.9

ACTIVATION OF K^+ CURRENTS IN CULTURED SCHWANN CELLS IS CONTROLLED BY EXTRACELLULAR PH D. Hoppe, H.-D. Lux, M. Schachner and H. Kettenmann
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K^+ currents recorded from cultured Schwann cells of mouse dorsal root ganglia were sensitive to changes in extracellular pH (pH_o). Currents were activated at potentials more positive than -50 mV, which is close to the resting membrane potential, and current amplitudes were affected by a change in (pH_o), being increased at alkaline and decreased at acidic pH_o. Analyzing the time course of current activation at different pH_o values indicated that the pH-sensitivity is due to changes in surface charges shifting the potential sensed by the gating process of the channel. The reversal potential of the currents was not affected by a change in pH_o. This observation and the finding that even a strong acidification to a pH_o value of 5.0 did not lead to a blockade of the fully activated channel indicate that the pH-sensitive charges are not located in the channel pore. Under the assumption that nerve activity in the peripheral nerve is associated with pH_o changes, as demonstrated for the optic nerve, the pH-sensitive K^+ channel of Schwann cells could serve to facilitate the spatial buffering of extracellular K^+.

396.10

REGULATION OF SCHWANN CELL K CHANNELS DURING INITIAL STAGES OF MYELINOGENESIS G.F.Wilson* and S.Y.Chiu. (SPON: T. Yin) Neuroscience Training Program & Dept. of Neurophysiology, University of Wisconsin, Madison, WI 53706.

In adult mammalian Schwann cells, ion channel expression is related to myelinogenic phenotype: K^+ currents are detectable at the soma of non-myelinating cells but not myelinating ones. We examine whether a developmental change occurs in K channel expression when the cell begins to form myelin. In cell-attached patches from myelin associated Schwann cells of rat sciatic nerve, delayed rectifying (DR) channels (~ 13-16 pS, 160 mM [K]o) are present in many somas as soon as myelin is visible (postnatal day 1, n=36). Inwardly rectifying (IR) channels also are observed and can be distinguished by their conductance (~ 35-37 pS, 160 mM [K]o), voltage dependence (IR channels being active at the resting potential), kinetics, and in inside/out patches, differential sensitivity to Cs^+ and 4-AP. Both IR conductance and reversal potential vary with external potassium, and in inside/out patches, rectification depends, in part, on [Mg^{++}]i. As myelination proceeds, somatic IR and DR channel density is down-regulated. The average IR current is reduced by 94% by day 8 (n=48) and the average DR current is reduced by 96% by day 45 (n=55), a time when IR channels have disappeared. This is the first report of an IR channel in normal mammalian Schwann cells. This, coupled with the observation that these channels are active at rest during rapid myelin formation may link them to myelinogenic processes.

Supported by NS-23375 (NIH), RG-1839 (National Multiple Sclerosis Society) and a PEW Scholar Award in Biomedical Sciences to S.Y.C.

397.1

INTERACTION OF GONADAL STEROIDS WITH $GABA_A$ RECEPTORS IN FRESH SLICES OF MEDIOBASAL HYPOTHALAMUS AND CEREBRAL CORTEX. W. Jacobson, D. March*, F Van Huizen*, M. Cynader, N. MacLusky and C. Shaw, Division of Reproductive Science, Toronto General Hospital and Department of Opthalmology, University of British Columbia.

GABA is the most ubiquitous neurotransmitter in the brain. It has been proposed that this neurotransmitter plays an important role in the regulation of LHRH release and ultimately gonadotropin secretion. We have examined the effects of a low dose regimen of gonadal steroids in ovariectomized females on $GABA_A$ receptors in slices of mediobasal hypothalamus (MBH) and cerebral cortex. Female Sprague Dawley rats which had been ovariectomized for 7 days were treated with estradiol benzoate (EB, 5 μg) or vehicle alone at 1000 hrs on day 0. On day 2, animals were treated with either progesterone (P, 500 μg) or vehicle alone at 1000 hrs. Animals were sacrificed at 1100 hrs, and $GABA_A$ receptor binding in slices of fresh brain tissue was determined using the $GABA_A$ antagonist [^{3}H]-SR-95531. Treatment with EB alone resulted in a decrease in the amount of [^{3}H]-SR-95531 bound in MBH. Subsequent treatment with progesterone resulted in a further decrease in [^{3}H]-SR-95531 binding in the MBH of a magnitude similar to that seen with EB treatment alone. In slices of cerebral cortex, binding determined with low concentrations of [^{3}H]-SR-95531 showed an identical pattern, however, when a high concentration of the radioligand was used the differences in binding between the three groups disappeared. We suggest that changes in behavior and in gonadotropin release normally seen in P treated, EB-primed animals may be reflective, in part, of a diminution in the inhibitory influence of GABAergic neural circuits. (Supported by a grant from the MRC of Canada to MC and from Wyeth Pharmaceuticals to WJ)

397.2

REGULATORY EFFECTS OF BARBITURATES AND STEROIDS ON THE $GABA_A$ RECEPTOR COMPLEX IN CULTURE. L. K. Friedman, T.T. Gibbs* & D.H. Farb, Dept. Anatomy & Cell Biol., SUNY HSC, Brooklyn, NY 11203.

Acute administration of barbiturates, benzodiazepines (BZDs) and steroids enhance GABA-mediated responses. Chronic BZD exposure reduces allosteric coupling between sites on the $GABA_A$ receptor. Here we report the effects of chronic barbiturate and steroid treatment of cultures derived from embryonic chick brain. Cultures were treated with barbital (1mM), pentobarbital (200μM), progesterone metabolites (5-ß-3-α- and 5-ß-3-ß-pregnan-ol-20-on; 10μM) or ß-estradiol (10μM) for 48 h. Allosteric interactions were measured by reversible binding with 1nM ^{3}H flunitrazepam in the presence and absence of 10μM GABA. Both barbiturates and steroids reduced allosteric coupling between GABA and BZD recognition sites, i.e. GABA's ability to enhance flunitrazepam binding was reduced by 30-50%. Steroids were more potent than barbiturates. Saturation analysis showed no change in receptor number. Direct enhancement of ^{3}H flunitrazepam binding by either barbiturates or steroids was abolished following chronic treatment. These results suggest that brain neurons in primary culture exhibit dynamic cellular regulatory responses to both barbiturates and steroids.

397.3

OPPOSITE EFFECTS OF PROGESTERONE ON GABA- AND GLYCINE-INDUCED CURRENTS IN CULTURED CHICK SPINAL CORD NEURONS. F.-S. Wu*, T.T. Gibbs* and D.H. Farb, Dept. of Anatomy and Cell Biology, SUNY Health Science Center, Brooklyn, NY 11203.

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

Using whole-cell recording methods cells were voltage-clamped at -70 mV. Drug solutions were applied to single neurons by pressure ejection from 7-barrel pipettes. Progesterone (10-100 μM) rapidly and reversibly potentiated responses to 3 μM GABA but reduced responses to 50 μM glycine. These effects on GABA and glycine responses were dose-dependent, with EC_{50}s of 26 and 15 μM, and maxima of +157% and -57%, respectively.

These results not only provide an interesting distinction between chloride-mediated GABA and glycine responses, but also suggest that endogenous progesterone may differentially modulate the inhibitory actions of these two neurotransmitters.

397.4

BINDING OF PREGNENOLONE SULFATE TO RAT BRAIN MEMBRANES: INTERACTION WITH THE $GABA_A$ RECEPTOR COMPLEX. S. Demirgoren* M.D. Majewska, H.N. Wagner, Jr.* and E.D. London (SPON: B. Gold), Addiction Res. Ctr., NIDA, Baltimore, MD 21224 and Division of Nuclear Medicine, The Johns Hopkins Medical Institutions, Baltimore, MD 21205.

The neurosteroid pregnenolone sulfate (PS) is synthesized in the CNS in oligodendroglia (Hu, Z.Y., et al., Proc. Natl. Acad. Sci., USA 84:8215, 1987). PS interacts with the $GABA_A$ receptor, presumably at the site of chloride ionophore, exhibiting GABA-antagonistic properties at micromolar concentrations (Majewska, M.D., et al., Brain Res. 339:178, 1985; Majewska, M.D. and Schwartz, R.D., Brain Res. 404:355, 1987; Majewska, M.D., et al., Neurosci. Lett. 90:279, 1988). We examined the binding of [^{3}H]PS to rat brain membranes. The binding of [^{3}H]PS was saturable and temperature- and protein-dependent; the optimal pH was 6.4 and equilibrium was reached after a 20 min incubation. At 5 nM, specific [^{3}H]PS binding was about 60% of total binding. A biphasic pattern of displacement of [^{3}H]PS by unlabeled PS suggested the existence of two populations of sites: Ki_1 = 2 μM and Ki_2 = 150 μM. Picrotoxin and antagonists of the $GABA_A$ receptor-coupled chloride ionophore inhibited [^{3}H]PS binding at the higher affinity site, suggesting that this site may be associated with the $GABA_A$ receptor complex. *In vitro*, PS interacted also with σ sites (Su, T.-P., et al., Science 240:219, 1988) but σ ligands (*d*-pentazocine and haloperidol) did not inhibit [^{3}H]PS binding, nor did progesterone, which also interacts with σ sites.

397.5

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

The effect of 5 days of food deprivation and refeeding for 5 days on GABA receptors, central benzodiazepine receptors (CBR), and peripheral benzodiazepine binding sites (PBS) was evaluated in adult female Sprague-Dawley rats in the estrous phase. Five days of food deprivation caused a significant reduction (20%) in body weight (control vs. deprived rats: 212±6 vs. 161±7 g; $p<0.01$). Starvation induced a significant down-regulation (30%; $p<0.01$) in PBS in the kidney. The alteration returned to normal values following 5 days of feeding. Ovarian PBS were not affected by food deprivation. [^{3}H]PK 11195 in the hypothalamus as well as the pituitary gland was unchanged. Starvation did not affect CBR binding to cerebral cortex, but it induced a significant decrease (35%; $p<0.01$) in [^{3}H]muscimol binding in this brain region. These results indicate that starvation stress affects GABAergic and benzodiazepinergic systems.

397.6

A PHYLOGENETIC STUDY OF THE EFFECTS OF UNSATURATED FATTY ACIDS ON THE GABA/BENZODIAZEPINE RECEPTOR COMPLEX Michael-Robin Witt and Mogens Nielsen, Research laboratories, Sct. Hans Hospital, Roskilde, Denmark.

Unsaturated fatty acids such as Oleic, Arachidonic and Docosahexaenoic acid stimulate the binding of the GABA/-Benzodiazepine receptor ligands 3H-Diazepam and 3H-Muscimol in rat brains in-vitro (Nielsen et al, Eur.J.Pharmacol.146,349,1988). In 6 species of mammals, Oleic acid (25 ul of a stock solution (4mM) was added to 1ml binding assay) increased the binding of these ligands by 100%. In the 9 species of birds studied, Oleic acid stimulated 3H-Diazepam binding to the same extent, while only weakly stimulating 3H-Muscimol binding (20%). The degree of 3H-Diazepam binding enhancement varied greatly depending on which of the 16 species of fish were studied. In some species such as coal fish, Oleic acid was inactive but stimulated the binding of 3H-Diazepam and 3H-Muscimol by 60% in other species (e.g. crucian carp). Modulation of the binding of 35S-TBPS by Benzodiazepine receptor agonist and inverse agonist ligands was less pronounced in e.g. the codfish, in contrast to the mammalian and avian species. Furthermore, incubations of brain membranes with Phospholipase A2 increased the binding of 3H-Diazepam in mammals and birds, while no increase was found in some fish brains (i.e. codfish). In conclusion, the allosteric interactions within the GABA/Benzodiazepine receptor complex differ between the species.

397.7

BENZODIAZEPINE-LIKE EFFECT OF A MONOCLONAL ANTIBODY ON PRESYNAPTIC GABA$_A$ RECEPTORS. B. Florán*, A. De Blas and J. Aceves* (SPON: J. Hernández). Dept. Physiology. CINVESTAV-IPN. Apartado Postal 14-740. 07000 México, D.F.

The aim of this study was to see how a monoclonal antibody raised for the purified GABA-A/benzodiazepine receptor could afect the activation of the presynaptic GABA receptors modulating the release of GABA in the pars compacta of the rat substantia nigra (Floran et al., Eur. J. Pharmacol. 150: 277, 1988). The experiments were done on slices of the nucleus. The release of [3-H] GABA was induced by high (15 mM) K^+. Muscimol inhibited GABA release (IC-50 = 35 nM). Both diazepam and pentobarbital caused a parallel shift to the left of the dose-response curve of the muscimol inhibition of the release. The monoclonal antibody (62-3G1) also shifted to the left the dose-response curve. The shifting of the curve by both diazepam and the antibody was blocked by the selective antagonist of the benzodiazepine receptors, Ro15-1788. The results suggest that the monoclonal antibody activates the benzodiazepine receptors of the GABA-A/benzodiazepine receptor complex increasing the affinity of the presynaptic GABA receptors for GABA agonists.

397.8

MODULATION OF CELL MITOGENESIS BY RO 5-4864 AND PK11195. H.E. Laird II, K.E. Gerrish* and D.H. Russell* Dept. of Pharmacol./Toxicol[1]., Univ. of Ariz., Tucson, AZ, 85721 & Pharmacol./Therap.[2], Univ. S. Florida, Tampa, FL.

We have used the Nb2 node lymphoma cell line as a model system in which the function of the PBZR may be studied. These cells possess high affinity PBZR sites and are dependent on prolactin (PRL) for mitogenesis. Ro 5-4864 (agonist) and PK11195 (antagonist) bind with high affinity to the peripheral benzodiazepine receptor (PBZR). We now report that Ro 5-4864 and PK11195 modulate the mitogenic activity of PRL in Nb2 cells. At 10^{-9}M, both Ro 5-4864 and PK11195 promoted PRL-directed cell proliferation while at 10^{-6}M only Ro 5-4864 inhibited the PRL effect. Further, Ro 5-4864 enhanced PRL-stimulated ODC activation at 10^{-9}M while 10^{-6}M inhibited the PRL effect. Next, studies were done to determine the effect of PK11195 on the Ro 5-4864 modulation of PRL. At 10^{-6}M, PK11195 blocked the PRL modulatory actions of 10^{-9}M and 10^{-6}M Ro 5-4864. In addition, Ro 5-4864 at 10^{-6}M blocked the ability of 10^{-9}M PK11195 to promote the mitogenic action of PRL. Finally, the presence of both agents at 10^{-9}M did not cause a greater effect on PRL-dependent mitogenesis than that shown by the agents individually. These data suggest that both ligands act on the same PBZR sites. Supported by Ariz. Dis. Contr. Comm.(YG-9290)(HEL) and Flinn Fdn. 062-100-099-86(HEL & DHR).

397.9

EXPRESSION OF GABA-RECEPTOR-LIKE IMMUNOREACTIVITY BY TYROSINE HYDROXYLASE CONTAINING HYPOTHALAMIC NEURONS IN DISSOCIATED CULTURE. **J. P. Grierson, H. M. Geller and H. D. Baker* Department of Pharmacology, UMDNJ - Robert Wood Johnson Medical School, Piscataway, NJ 08854. *Dept. of Neurology, Burke Rehabilitation Center, Cornell University, White Plains, NY 10605.**

The role of GABA as a neuroendocrine transmitter in the hypothalamus is a subject of considerable investigation. Especially interesting are putative interactions between GABA and dopaminergic neurons. Our previous immunocytochemical characterization of dissociated neurons from the embryonic rat hypothalamus in culture have shown two populations of cells immunoreactive for tyrosine hydroxylase (TH) and also that the majority of neurons are immunoreactive for GABA. We have also shown that the majority of neurons in our culture express GABA/benzodiazepine receptor (GABAr) immunoreactivity. In this study, we wanted to ascertain whether TH-containing cells express the GABAr. Cultures were fixed in a 4% paraformaldehyde (PFA) solution and then incubated with the monoclonal anti-GABAr antibody 6AA2 (J. Tallman), followed by rhodamine conjugated secondary antibody. A second PFA fixation and permeabilization with Triton was completed before staining with rabbit anti-TH (EugeneTech) and a fluorescein-conjugated secondary antibody.

The antibody 6AA2 possessed a highly characteristic staining pattern on cultured neurons with intensely stained plaques in restricted areas. These plaques are normally located on the neurites, although partial somatic staining is occasionally observed. Approximately 0.1% of neurons stain brightly for TH and initial results indicate that all have a few GABAr-positive plaques. Thus, our data provide direct evidence for a GABAergic modulation of dopaminergic neuronal activity. Supported by NIH NS-25168.

397.10

CENTRAL AND PERIPHERAL GABA RECEPTORS IN THE COCKROACH PERIPLANETA AMERICANA. M.E. Schnee*, S.D. Buckingham*, J.J. Rauh*, B. Hue*, M.E. Schroeder* and D.B. Sattelle (SPON: J. Meek). Agricultural Products Dept., E.I. DuPont de Nemours, Wilmington, DE 19898 and AFRC Unit, Dept. of Zoology, Cambridge, U.K.

Previous attempts to classify insect GABA receptors have lacked comparative information for central versus peripheral receptors. Our electrophysiological studies on identified neuronal cell bodies of the cockroach have revealed the presence of bicuculline-insensitive GABA-activated chloride channels. In contrast, bicuculline (100 μM) blocked IPSPs recorded from giant interneurone 2 (GI 2) following stimulation of nerve X, but failed to block the response to GABA ionophoresed onto the fine branches of GI 2 within the neuropile of the terminal abdominal ganglion. Thus, the block by bicuculline of IPSPs recorded from GI 2 resulted from an action at sites other than postsynaptic GABA-activated chloride channels. Bicuculline blocked neuronal nicotinic ACh receptors in cockroach CNS, which may explain the observed synaptic effects. Our preliminary findings indicate that bicuculline-insensitive receptors are also present on cockroach coxal muscles. Current studies are aimed at further electrophysiological characterization of these peripheral receptors in comparison to central insect receptors and to ligand binding profiles obtained from membrane preparations of the cockroach ventral nerve cord.

397.11

PHARMACOLOGICAL DIFFERENCES BETWEEN GABA RECEPTORS OF INSECTS AND VERTEBRATES. S.C.R. Lummis*, S.D. Buckingham*, J. Rauh* and D.B. Sattelle. AFRC Unit, Dept of Zoology, Cambridge, U.K. and Agricultural Products Dept, E.I. Du Pont de Nemours, Wilmington DE, U.S.A.

GABA receptors in the insect CNS show biochemical similarities to the vertebrate GABA$_A$ receptor, but their pharmacological properties differ. Electrophysiology, radiolabelled ligand binding and GABA-activated $^{36}Cl^-$ uptake have been used to study the actions of a variety of ligands at these receptors. Cockroach CNS contains a GABA receptor which is insensitive to bicuculline and possesses a benzodiazepine binding site more closely resembling that of the vertebrate peripheral benzodiazepine binding site. Selected steroids with IC_{50s} of 10-1000nM against ^{35}S-TBPS binding to rat brain have little or no effect on the insect GABA receptor; the most active (5α-Pregnan-3α-OL-20-ONE) inhibits only 26% of cockroach CNS GABA-stimulated $^{36}Cl^-$ uptake at 1μM. The cyclodiene heptachlor has an IC_{50} of 0.19μM against vertebrate ^{35}S-TBPS binding, but on cockroach CNS at 100μM it only blocks up to 50% of the electrophysiological response to GABA and 55% of the CNS GABA-stimulated $^{36}Cl^-$ uptake. We conclude that there are differences between insect and vertebrate GABA receptor Cl^- channel molecules at the sites of action of bicuculline, benzodiazepines, steroids and heptachlor.

397.12

EFFECT OF PRENATAL AND NEONATAL CHRONIC EXPOSURE TO PHENOBARBITAL ON CENTRAL AND PERIPHERAL BENZODIAZEPINE RECEPTORS. F. Fares,* A. Weizman,* C. G. Pick,* J. Yanai* and M. Gavish. Dept. of Pharmacology, Technion Fac. of Med., 31096 Haifa, Israel. (SPON: N. Allon)

Phenobarbital (PhB) was administered to mice during days 9-18 of gestation. [^{3}H]Muscimol binding to cerebellum, [^{3}H]flunitrazepam binding to cerebellum and olfactory bulb, and [^{3}H]PK 11195 binding to olfactory bulb, heart, and kidney were assayed in the offspring at 22 and 50 days of age. The chronic prenatal administration of PhB did not affect either GABA receptors, central benzodiazepine receptors (CBR), or peripheral benzodiazepine binding sites (PBS) in these tissues. In the next stage of the study, we investigated a possible modulatory effect of chronic postnatal PhB treatment during days 2-21 of age on the same receptors measured at 22 and 50 days of age. PhB exposure of neonates resulted in a significant down-regulation of GABA receptors and CBR in the cerebellum and of PBS in the heart. The effects were demonstrated on day 22 of age and were undetectable by day 50 of age. CBR at the olfactory bulb and PBS at the olfactory bulb and kidney were not altered by the drug treatment.

397.13

AHR-14749: A NEW BZ_1 BENZODIAZEPINE RECEPTOR SELECTIVE ANXIOLYTIC/ANTICONVULSANT. B. F. Kilpatrick, R. D. Tabor*, R. Young, B. E. Tomczuk*, C. R. Taylor* and D. N. Johnson. A. H. Robins Research Laboratories, Richmond, VA 23261.

Biochemical and pharmacological characterization of AHR-14749 (5-chloro-N,N-dimethyl-2-(4-methylphenyl)-3H-imidazo[4,5-b]pyridine-3-acetamide) indicates that this compound is selective for the BZ_1 benzodiazepine receptor and possesses potent anxiolytic and anticonvulsant activities. Competition curves of AHR-14749 for [^{3}H]flunitrazepam (FLN) binding in rat cerebral cortex membrane preparations were shallow with Hill coefficients of 0.634 and computer analysis indicated two sites with K_i values of 41 and 1400 nM. Analysis of AHR-14749 competition for [^{3}H]FLN binding to the BZ_1 receptor in rat cerebellum revealed a single site with a K_i value of 59 nM indicating that AHR-14749 had a 30 fold selectivity for the BZ1 receptor. GABA and barbiturate shift ratios of 2.75 and 2.65 indicated that AHR-14749 behaved as a full agonist and scatchard analysis indicated that AHR-14749 was a competitive inhibitor of BZ_1 receptors.

In vivo studies showed that AHR-14749 was an effective anticonvulsant as indicated by its ability to inhibite maximal electroshock-induced (ED_{50}=9.1 mg/kg,IP) and subcutaneous Metrazol-induced (ED_{50}=2.98 mg/kg,IP) convulsions in mice. AHR-14749 was also shown to be a potent anxiolytic as indicated by the minimum effective doses in the Vogel (3.16 mg/kg,IP) and in the light/ dark exploratory (0.10 mg/kg,IP) behavioral tests in mice. Using blockade of morphine-induced Straub Tail (ED_{50}=0.87 mg/kg,IP) and the rotorod motor performance (ED_{50}=17.8 mg/kg,IP) tests, AHR-14749 was shown to have less muscle relaxant activity than diazepam. Using values derived from the light/dark exploratory and Straub Tail tests, AHR-14749 had a 75 times better anxiolytic/muscle relaxant therapeutic index than diazepam.

397.14

KINDLING PRODUCED IN RATS BY PENTYLENETETRAZOL (PTZ) IS ASSOCIATED WITH A DECREASE IN THE GABA-STIMULATED 36Cl- UPTAKE IN THE CEREBRAL CORTEX. M.G. Corda, B. Longoni*, M. Orlandi*, D. Lecca*, E. Cancedda* and G. Biggio. Dept. of Exp. Biology, Univ. of Cagliari, Italy

We have previously shown that chronic treatment with FG 7142 produces chemical kindling in rats, and this effect is associated with a down-regulation of the GABA-A receptor complex (Brain Res. 384, 60, 1986). To further clarify the role of the GABAergic system in the development of chemical kindling, in the present study we evaluated in rats the sensitivity to convulsions and the function of the GABA-A receptor complex following chronic treatment with PTZ, a blocker of the GABA-coupled chloride ionophore. Repeated administration of subconvulsant amounts of PTZ (25 mg/kg i.p., 3 times a week for 10 weeks) resulted in the gradual development of kindling. Convulsions appeared by the 2nd-3rd week of treatment and 90-100% of the rats became sensitized to PTZ by the end of the chronic treatment. Sensitization to convulsions was long-lasting, being still present in rats challenged with PTZ (25 mg/kg i.p.) up to 4 months after the completion of the chronic treatment. In addition, rats kindled with PTZ showed an enhanced responsiveness to FG 7142 (30 mg/kg i.p.), Ro 15-4513 (20 mg/kg i.p.) and isoniazid (300 mg/kg s.c.). Biochemical studies, performed 3 days after the completion of the chronic treatment (10 weeks), revealed a reduction in the stimulatory effect of GABA on 36Cl- uptake to cerebral cortex microsacs and a decrease in the density of 35S-TBPS binding sites in unwashed cerebral cortex membranes from PTZ-treated rats. In addition, a significant increase in the total number of BZD receptors was observed in the cerebral cortex and hippocampus, but not in the cerebellum of rats kindled with PTZ. The results suggest that kindling produced by repeated exposure to PTZ is associated with a reduction in the function of the GABA-coupled chloride ionophore.

397.15

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

Studies examining the relationship between perinatal exposure to diazepam (DZ) and subsequent alterations in central benzodiazepine receptors have yielded inconsistent results. We have examined the effect of perinatal exposure to DZ on the low affinity GABA A receptor in the adult rat.

Rats were exposed to DZ from embryonic day 8 to postnatal day 7 (P7). Animals were sacrificed at P90 and several mesotelencephalic brain regions were removed. The binding of the GABA antagonist, [^{3}H]bicuculline methyl chloride (BMC), to P_2 membranes in potassium phosphate/thiocynate buffer was measured.

A significant decrease in BMC binding was found in cingulate cortex membranes from animals exposed to DZ, relative to untreated controls. In addition, the ability of GABA to displace BMC binding was significantly reduced in hypothalamus membranes from DZ-exposed subjects. Our results suggest that some of the behavioral and biochemical alterations previously seen in animals perinatally exposed to DZ may be associated with an alteration in the GABA A receptor. This work was supported in part by MH 14092 and the Abraham Ribicoff Research Facilities of the Connecticut Mental Health Center.

397.16

GABA MEDIATED CHLORIDE FLUX IS NOT ALTERED DURING MAXIMAL BENZODIAZEPINE (BZ) WITHDRAWAL (WD). Norman R. Boisse, Yu Xie, and Gary M. Samoriski, Sect. of Pharmacology, Northeastern University, Boston, MA 02115.

Chronic BZ anxiolytic-sedative-hypnotics can induce physical dependence. The mechanisms of WD are unknown. Spinal cord electrophysiological studies revealed GABA-mediated pre-synaptic inhibition is profoundly reduced during max. BZ WD in a rebound way (JPET 231: 464, 1984) supporting a GABA hypoeffectiveness hypothesis. To test whether the GABA-BZ receptor Cl channel complex is a locus for GABA transmission deficits, brain microsac GABA stimulated ^{36}Cl flux (Harris & Allan, 1985) was evaluated during WD. Rats got chlordiazepoxide (CDP) 150 mg/kg, b.i.d. or H_2O (controls) for 5 weeks; 3 1/2 days, at max WD flux was done. There was no change in the GABA dose-response. CDP and pentobarbital increased GABA flux 5% and 46% in both groups. Therefore, GABA receptor-Cl channel coupling is not altered in BZ WD. Disparity with in situ spinal reflex study suggests endogenous modulators of the GABA receptor complex, intact synaptic transmission and/or reduced GABA release may play a role in WD expression (BRSG S07 RR 05830-09).

397.17

DECREASED PERIPHERAL BENZODIAZEPINE BINDING CAPACITY IN DEPRESSED PATIENTS. D.L. Diorio, S.A. Welner, B.E.Suranyi-Cadotte Douglas Hosp. Research Center, Department of Psychiatry, McGill Univ., Montreal, Canada H4H 1R3

Peripheral benzodiazepine (PBZ) binding sites are widely distributed in peripheral tissues as well as in the CNS, however they are pharmacologically and physically distinct from the central benzodiazepine receptors which mediate the anxiolytic, sedative and anti-convulsant actions of the classical benzodiazepines. Although the precise function of the PBZ site is as yet unclear, a role in the stress response has been suggested since variable changes in the PBZ sites have been observed in peripheral and central tissues of animals exposed to stress. Additionally, the PBZ site may reflect changes occurring in anxiety, since a reduced binding capacity (Bmax) has been reported in platelets of anxious patients (Weizman et al., Eur. J. Pharm., 138:289, 1987). Due to the intimate relationship existing between anxiety and depression, we investigated the binding of [^{3}H]PK 11195 (a ligand highly specific for the PBZ site) to platelets in a group of depressed patients (RDC-major depression) and healthy controls matched for sex and age. We report here a significant decrease in Bmax values in depressed patients (2905.5±514.2) when compared to controls (4235.9±477.3); no change in the affinity (Kd) of the ligand was observed. These results provide further evidence suggesting a common biological component between depression and anxiety, where similar changes in PBZ receptor densities have been previously observed. Supported by FCAR & FRSQ.

397.18

DISCIMINATIVE STIMULUS EFFECTS OF MIDAZOLAM (MDZ) AFTER CENTRAL ADMINISTRATION IN RATS. C.A. Sannerud and R.R. Griffiths. Depts. of Psychiatry and Neuroscience, Johns Hopkins Univ. Sch. of Medicine, Baltimore, MD 21205.

Since the drug discrimination procedure has been shown to provide a highly specific assessment of the pharmacological actions of the benzodiazepines (BZ), a drug discrimination paradigm was used to investigate central sites of BZ activity. Male Long-Evans hooded rats were trained to discriminate 0.32 mg/kg MDZ i.p. vs. no drug (ND) in a two-lever drug discrimination procedure. Rats were implanted with bilateral intracranial cannulae in the basolateral amygdala and lateral ventricle. On selected test days, intracranial microinjections of MDZ was delivered into the specific brain regions of unrestrained rats. Compared to the i.p. route, central administration of MDZ in either brain area was 4-times more potent in producing increases in drug-lever responding (ED_{80}: 8 vs. 32 µg). Drug-lever responding occasioned by intracranial MDZ injections in the lateral ventricles or basolateral amygdala was antagonized in a dose-dependent manner by intraperitoneal administration of the BZ receptor antagonist, flumazenil. The antagonism by flumazenil was surmountable with a higher intracranial dose of MDZ. Thus, although no site specificity was demonstrated, these data suggest that the drug stimulus produced by MDZ administration was mediated via central BZ receptors. Supported by DA-04133.

398.1

GABA$_A$-RECEPTORS EXPRESSED FROM RAT α- AND β-SUBUNITS IN XENOPUS OOCYTES ARE MODULATED BY BENZODIAZEPINE RECEPTOR LIGANDS.

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(SPON: J.R. Martin)

The structural requirements for functionally active GABA$_A$-receptors were investigated in Xenopus oocytes by coexpression of clones coding for the α- and β- subunits of the receptor in rat brain. GABA-induced inward currents, recorded under voltage clamp at -70mV, were selective for chloride ions. They were inhibited by bicuculline and potentiated by pentobarbital as well as by diazepam (1-20 μM) and flumazenil (1-10 μM). Surprisingly, the inverse agonist DMCM (3-10 μM) likewise potentiated the GABA-response. Thus, benzodiazepine binding sites are present on GABA$_A$-receptors expressed from rat brain α- and β- subunits. They mediate, however, only a limited spectrum of intrinsic activities. The findings are in line with the biochemical localization of benzodiazepine binding sites on α-subunits of the native GABA$_A$- receptor.

398.2

THE LONG-TERM, CONTINUOUS RELEASE OF FG 7142 INCREASES GABA-STIMULATED CHLORIDE UPTAKE IN CORTICAL MEMBRANE PREPARATIONS.

R. J. Marley, C. Heninger and D. W. Gallager. Dept. of Psychiatry, Yale U. Sch. Med., New Haven, CT 06508.

Repeated, intermittent administration of the β-carboline, FG 7142, results in chemical kindling with a concomitant increase in seizure susceptibility (Little et al., Br. J. Pharm., 83:951, 1984). This treatment also produces an apparent decrease in the number of GABA and TBPS bindings sites and a decreased ability of muscimol to stimulate the uptake of $^{36}Cl^-$ into cortical membrane preparations (Corda et al., Brain Res. Bull., 19:379, 1987; Lewin et al., Eur. J. Pharm., 160:101,1989). We have previously reported a similar decrease in GABA-stimulated $^{36}Cl^-$ influx into cortical membrane preparations following the continuous, long-term administration of diazepam (Marley, R.J. and D.W. Gallager,. Eur. J. Pharm., 159:217, 1989). However, both kindling and the development of tolerance to benzodiazepines appear to be dependent on whether the drug is administered intermittently or continuously.

In the present study, FG-7142 was administered continuously for 10 days using osmotic minipumps modified with catheters which were stereotaxically implanted to release drug directly into the ventricles. This protocol allows for the administration of 50 μg/day over the entire treatment period. Cortical membrane preparations from rats administered FG 7142 continuously for 10 days exhibited an increase in GABA-stimulated $^{36}Cl^-$influx, suggesting that while the intermittent administration of FG 7142 leads to biochemical changes associated with kindling, the continuous administration of this inverse agonist has an opposite effect. This effect is also opposite in direction to that observed following chronic agonist exposure. These results indicate that the GABA/benzodiazepine receptor complex may homeostatically respond to continuous modulation by benzodiazepine ligands.

Further results from determinations of seizure susceptibility, the binding of TBPS to the chloride channel and GABA receptor binding parameters following continuous ICV administration of FG 7142 will also be presented.

398.3

ENANTIOSELECTIVITY IN THE POTENCY OF AGONISTS AT THE FUNCTIONAL GABA$_A$ RECEPTOR.

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Membrane vesicles from the cerebral cortex of male Wistar rats were prepared for both binding studies and $^{36}Cl^-$ influx measurements. All experiments were performed under identical conditions (30° in Hepes buffered physiological salt solution pH 7.5, 2.5 mg protein/mL); incubation for binding was 30 min, Cl^- uptake was terminated after 7 sec by mixing with a quench-solution containing picrotoxin and furosemide (3 mM each). S(+)-dihydromuscimol (DHM) was about 6 times as potent as R(-)-DHM both in displacing specific ^3H-muscimol binding and in the activation of $^{36}Cl^-$ influx. S(-)-Ro11-3128 was found over 100 times more potent than R(+)-Ro11-3624 both in displacement of specific ^3H-flunitrazepam binding and in the facilitation of $^{36}Cl^-$ influx. Moreover, the potent effect of DHM enantiomers appeared at the same concentration in both experiments (0.7 uM for S(+)-DHM, 4 uM for R(-)-DHM) suggesting the presence of GABA$_A$ receptors exhibiting similar potencies in both binding and functional response.

398.4

GABA DESENSITIZATION IN HIPPOCAMPAL NEURONS IN CULTURE.

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Application of GABA to hippocampal neurons in culture (WC mode with symmetric Cl concentrations) via local perfusion, results in a dose-dependent desensitizing inward current ($\tau \cong 6$-7 seconds). Desensitization may be present during ongoing synaptic activity, as suppression of neurotransmitter release with Co increases the peak response to GABA and the desensitization rate while decreasing the steady state response.

We attempted to determine if the rate and extent of desensitization to GABA could be affected by a cAMP- or G protein-dependent process or modification of the high affinity GABA binding site. Neither internally perfusing the neurons with diB-cAMP, nor treatment of the cultures with pertussis toxin (to persistantly inactivate G proteins), nor intracellular perfusion with the non-hydrolysable substrate, GTPγS, dramatically altered the size of the GABA response or its desensitization. Addition of Mg and ATP to the patch pipette slowed "run-down" of the GABA responses but did not affect desensitization. Application of SCN, which has been suggested to mask high affinity GABA binding sites, decreased both peak and sustained currents without affecting the rate of desensitization.

We also examined the effects of somatostatin-14 and 28, peptides which commonly coexist with GABA in hippocampal neurons, on the GABA response. SOM-14 (100 nM) enhanced the peak GABA response in ca. 40% of neurons, and decreased it in ca. 15%. SOM-28 (100 nM) increased the GABA response in 9% of neurons and decreased it in 36%. Neither form of SOM appeared to have a significant effect on the extent or rate of desensitization. Thus, both forms of SOM can modulate the postsynaptic GABA receptor, but their effects are variable from cell to cell and can be positive or negative.

398.5

GABA ACTIVATED CURRENTS AND CHANNELS IN MAMMALIAN NEOCORTICAL NEURONS.

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Responses to GABA were analyzed in neocortical neurons recorded with WC patch clamp electrodes. Only $GABA_A$-Cl currents were activated; there were no currents which could be attributed to $GABA_B$ receptors. The GABA response decremented with maintained GABA due to two factors: changes in internal Cl (when Cl_i was initially low) and true receptor desensitization. Both desensitization and resensitization were voltage sensitive; at hyperpolarized membrane potentials, the neurons desensitized relatively rapidly (≅ 10-15 sec) and resensitized slowly. At positive membrane potentials, desensitization was slow and resensitization was rapid.

When GABA-activated Cl channels were examined in outside-out patches, desensitization occurred rapidly and was no longer voltage dependent. Moreover, a very rapid "run-down" of the channel responses was noted. Thus, the response in outside-out patches was quite different from that of the same channels in the WC mode. The channels averaged 27 pS in their main conductance states, but occasional 16 pS and 45 pS channels were noted. I-V curves were linear for all of the 27 pS channels examined.

Preliminary experiments were performed to identify regulatory mechanisms underlying the desensitization or resensitization of the WC responses. Neither addition of Mg and ATP, nor cAMP, cGMP, ATP or GTP, to the patch pipette, nor changes in levels of extra- (2 or 10 mM) or intracellular Ca (1 nM to 10 uM - buffered with EGTA), nor treatment of the cultures with forskolin or phorbol 12-myristate-13-acetate (PMA) produced a change in either the GABA response or its voltage dependent desensitization or resensitization.

398.6

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

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GABAergic inhibition develops primarily after birth, has been shown to be decreased following some forms of chronic deafferentation, and may decline over time in culture (McBain *et al.* 1988). We therefore used immunohistochemical staining with an anti-GABA antibody, and intracellular recording to examine the GABAergic system in hippocampal roller-tube slice cultures. Interneurons and terminal-like elements containing GABA-immunoreactivity had a similar morphology, distribution, and density to *in situ* tissue, and there was no loss of immunoreactivity in cultures kept for 13-26 days *in vitro*. Furthermore, after 8-30 days in culture, spontaneous and evoked IPSPs were observed in all CA3 pyramidal cells tested. These IPSPs resulted from an increase in Cl- conductance, and were mediated by activation of bicuculline-sensitive GABA receptors. Both the magnitude of the synaptic conductance underlying the maximal IPSP (mean = 130 nS) and the IPSP reversal potential (-66 mV) were comparable to *in situ* values, and did not vary with time in culture. Epileptiform bursting was not observed under control conditions. Taken together, the present physiological and immunohistological data show that GABAergic inhibition is well expressed in organotypic slice cultures and is maintained over periods of at least four weeks *in vitro*. Supported by Swiss National Fund grants 3.389.86, 3.390.86, and 3.534.86 (PS and BHG) and NIH Fellowship F32 NS08306 (SMT).

398.7

THE PUTATIVE ROLE OF Ca^{2+} CHANNELS IN THE CALCIUM-INDEPENDENT RELEASE OF GABA. S. Bernath and M.J. Zigmond. *Department of Behavioral Neuroscience and Center for Neuroscience, University of Pittsburgh, Pittsburgh, PA 15260.*

We have investigated whether external Ca^{2+} is crucial for GABA release from striatum. Tissue slices (350 um) were prepared from rat striatum, incubated with [3H]GABA, superfused with Krebs buffer (100 ul/min), and exposed to electrical field depolarization (2 Hz for 3 min). Tritium efflux was measured as an index of GABA release. Removal of external Ca^{2+} (and addition of 1mM EGTA) greatly increased resting and evoked efflux of GABA. However, there was no effect of Ca^{2+}-deficiency on resting release when Mg^{2+} was elevated to 10 mM, and evoked overflow was depressed (-88%). Verapamil (10 uM), a Ca^{2+} channel blocker, reduced evoked overflow under normal conditions (-45%) and in the absence of Ca^{2+} (-85%). 4-Aminopyridine (10 uM), a K^+ channel inhibitor, increased GABA release in the presence of external Ca^{2+} (+78%), but not in its absence. These results suggest that Ca^{2+} channels may play a double role in the regulation of GABA release, only one of which involves Ca^{2+} influx. (Supported in part by USPHS grants NS-19608, MH-43947, and MH-00058.)

398.8

AN EXCITATORY RESPONSE TO GABA IN CRUSTACEAN MOTONEURONS. R. M. Harris-Warrick and J-R Cazalets* Section of Neurobiology and Behavior, Cornell University, Ithaca NY 14853.

γ-aminobutyric acid (GABA) is normally thought of as an inhibitory neurotransmitter. However, in several invertebrate and vertebrate systems, GABA has now been shown to exert excitatory effects on neurons. Here we show that in the lobster, *Homarus americanus*, GABA elicits a depolarizing response in the pyloric motoneurons of the stomatogastric ganglion. These responses are excitatory and result in action potential generation. The depolarization is preferentially elicited by pressure application on the cell bodies, which can be studied by two-electrode voltage clamp. The response is dose-dependent with a threshold at 10^{-5} M and saturation at 10^{-3} M. The excitatory effect is mimicked by muscimol and blocked by picrotoxin but not by bicuculline. The response is TTX-, TEA- and 4-AP-resistant. V_{rev} is between -30 and -20 mV and is not altered by changes in extracellular K^+ and Ca^{2+} (from 20% to 400% of the normal concentration). Decreases in extracellular Na^+ cause a negative shift of V_{rev} while decreases in extracellular Cl^- produce a positive shift. We conclude that the GABA depolarizing response is a mixed response caused by conductance increases to Na^+ and Cl^- ions. Supported by NIH NS17323 and Hatch NYC191410.

398.9

DIFFERENTIAL EFFECT OF HALOPERIDOL ON GAD ACTIVITY IN DISCRETE BRAIN NUCLEI IN THE RAT. R. E. Wilcox, P. K. Randall, and R. D. Mayfield*. Institute for Neuroscience, Depts. of Pharmacol. and *Kinesiol., Univ. of Texas at Austin, Austin, TX 78712

It has been suggested that different motor behaviors produced by dopamine (DA) agonists and antagonists may be mediated though different striatal efferent systems. Further, different efferents may be characterized by distinct relationships with the DA receptor subtypes. If this is the case, experimental manipulations of striatal output should differentially affect the neurochemical characteristics of the output nuclei. We studied the effect of acute DA receptor blockade on glutamic acid decarboxylase (GAD) activity in the major striatal output nuclei.

Male Sprague-Dawley rats (n=33) were administered the predominantly D-2 antagonist haloperidol (0.5 or 5 mg/kg, ip) or 0.01M tartaric acid (vehicle). Micropunches were taken from the substantia nigra reticulata (SNr), globus pallidus (GP) and the entopeduncular nucleus (EP) and assayed for GAD activity using the method of Holdiness (Anal. Lett., 13: 1330, 1980). Acute challenge with 0.5 and 5 mg/kg haloperidol decreased GAD activity in the SNr by 24 and 59%, respectively. This dose dependent decrease was not observed in the GP or EP. Haloperidol produced a biphasic effect on GAD activity in both the GP and EP, with the effect being more pronounced in the EP. The 0.5 mg/kg dose of haloperidol decreased GAD activity by 30 and 77% in the GP and EP, respectively. The 5 mg/kg dose did not significantly affect GAD activity in the GP; however, it produced a 45% decrease in the EP. Thus, in GP and EP, the low dose of haloperidol was more effective in decreasing GAD activity than the high dose.

In conclusion, brain regional GAD activity may represent a useful approach for assessing the effects of typical and atypical neuroleptics on basal ganglia efferent activity. Comparisons of these results with those of nonselective or selective D-1 antagonists would be valuable in assessing the contributions of DA recepor subtypes to the function of striatal efferent systems. (Supported by NS20827, MH44799, BN58808129, NS22926, and the Texas Adv. Res. Tech. Prog.)

398.10

ONTOGENY OF GABA LOCALIZATION IN PAROXYSMAL CHICK BRAIN. P. A. Lewis* and M. M. Beck. Dept. of Animal Sci., Univ. of Nebraska, Lincoln, NE 68583.

GABA, an inhibitory neurotransmitter and metabolic substrate in brain, has been implicated in seizure syndromes, increasing in some (Roberts et al., 1985), decreasing in others (Ciesielski et al., 1981). In 10-20d paroxysmal (px) chicks, it increases (Firman and Beck, 1984). The px syndrome includes clonic-tonic audiogenic seizures in chicks beginning at 7-10d posthatching; seizures can also be electrically elicited by 5d. In this study, brains from px and normal chicks were removed at 5, 7, and 10d and stained for GABA using immunocytochemistry. Cells stained at 7 but not at 5d in 5 oculomotor and 2 auditory regions. Intensity of optical density staining was from 50-77% greater in 2 auditory and 1 oculomotor areas in px brains at 7d. Increased terminal staining in auditory nuclei was found in normal brains compared to px brains of the same age but increased over time in both groups. At 10d, cells in px cerebellum were more intensely stained than the 7d px or 10d normals. The results indicate accumulation of GABA in cells and decreases in terminals in px brain, possibly because of decreased transport along degenerated auditory tracts (Beck et al., 1983) or to an increase in glial metabolic pools.

398.11

GABA TERMINALS IN THE MOUSE DENTATE FASCIA LABELED WITH IMMUNOGOLD PROBE. P. Schaner* and E. Fifkova (SPON: T. D. McIntyre). Neuroscience Center, Department of Psychology, University of Colorado, Boulder, CO 80309.

Distribution of GABAergic terminals in the dentate fascia of mice was studied with the immunogold probe. Thin sections were incubated with an affinity purified antibody against GABA and then labeled with a 5 nm gold-conjugated secondary antibody.

Two types of labeled terminals are found in the granule cell layer synapsing upon the granule cell bodies and large dendrites. The first type, an asymmetrical synapse, is identified by a distinct postsynaptic density and numerous, tightly packed, large round vesicles. The second type, a symmetrical synapse, lacks the thick postsynaptic density but often exhibits presynaptic densities along the synaptic apposition. The vesicles are less uniform in size and appear loosely packed, except along the active zone, where they are tightly clustered. Preliminary results indicate that asymmetrical synapses occur more frequently (61% of those analyzed) than the symmetrical type. Dense core vesicles are found in both types. The relatively large proportion of asymmetrical GABAergic synapses is an unexpected finding. It is unlikely that the GABA antibody used cross-reacted with another transmitter metabolically close to GABA, like glutamate, because in the distal two-thirds of the dentate molecular layer where glutamatergic synapses form more than 85% of the total synaptic population labeled terminals are rare. Asymmetrical GABAergic terminals labeled with an anti-GABA antibody and a gold probe were also observed in the cerebellar glomeruli (Hamori and Takacs, *Exp. Brain Res.*, **74**:471, 1989). However, our observation is at variance with that of Kosaka et al. (*Brain Res.*, **293**:353, 1984) who have found that in the rat granule cell layer less than 1% of GABAergic terminals are asymmetric. Whether this discrepancy is caused by the difference in the procedure (GAD antibodies visualized with HRP immunocytochemistry in a preembedding mode) or in the species used will require further analysis.

Supported by NIAAA Grant AA06196.

398.12

IMIDAZOLEACETIC ACID, A GABA-A AGONIST, AND ITS CONJUGATE(S) IN CNS OF RAT: HETEROGENEOUS DISTRIBUTION AMONG REGIONS, SUBCELLULAR FRACTIONS AND CEREBROSPINAL FLUID. G.D.Prell, A.M.Morrishow* and J.P.Green. Dept. Pharmacology, Mount Sinai School of Medicine, CUNY, New York, NY 10029

Imidazoleacetic acid (IAA), which we demonstrated in rat brain and human CSF by GC-MS (J.Neurochem. 52:1107,1989), stimulates GABA-A receptors, stimulates phosphodiesterase activity and produces analgesia, hypnosis, hypothermia and hypotension. In brains of rats that had been anesthetized and cerebrally perfused to remove blood that contains IAA, levels of IAA are distributed heterogenously among regions. Mean (±SEM) levels (pmol/g) ranged from 291±47 (olf. bulbs) to 105±8 (frontal cortex), a three-fold difference (ANOVA: $F=14.8$, $p< 0.01$). Levels of IAA were heterogenously distributed (mean % of total) among the soluble (63%), P2 (mitochondrial)(23%), P1 (nuclear)(9%) and P3 (microsomal)(5%) fractions; levels in soluble and P2 fractions were negatively correlated ($r= -0.98$, $p<0.0001$). There was a concentration gradient of IAA in samples of CSF taken sequentially from the cisterna magna of anesthetized rats, i.e. last units of CSF collected had higher levels than portions removed earlier, suggesting that, like other substances showing concentration gradients, IAA in CSF may be derived from brain. We found high concentrations of an acid-hydrolyzable conjugate(s) of IAA in brain (> 20 nmol/g) and CSF, also heterogenously distributed, whose levels exceeded those of free IAA by up to 100-fold. Levels of IAA in homogenates heated at 100°C for 48 h in 2N HCl were unchanged, but increased after heating at 150°C in 0.1N HCl. These findings suggest that the conjugate(s) is likely to be IAA-ribotide or IAA-riboside, and its formation may be a means of terminating the actions of IAA. IAA in brain may be derived physiologically from histidine with imidazolepyruvic acid as an intermediate since IAA levels increased in rats after irrevesible inhibition of histidine decarboxylase and administration of histidine and increased in brain homogenates incubated with imidazolepyruvic acid. IAA may also be formed in brain from large, nonphysiological quantities of histamine. [Supported by NIMH grant 31805.]

398.13

AFFERENTS TO CALCIUM-BINDING PROTEIN CONTAINING BASAL FOREBRAIN NEURONS. W.E. Cullinan and L. Zaborszky. Dept. Otolaryngol. Univ. of Virginia, Charlottesville, VA 22908.

Parvalbumin (PV) and calbindin D-28k (CaBP) are calcium-binding proteins (CBP) important in sequestering free intracellular calcium. Antibodies against these proteins are thought to label two distinct populations of GABAergic neurons without the use of colchicine. Earlier studies (Zaborszky et al., 1986) have shown that local or projective GABAergic afferents in the basal forebrain innervate cholinergic projection neurons. Since little is known about afferents to basal forebrain GABAergic neurons, we were particularly interested in identifying afferents to CBP neurons in basal forebrain areas rich in cholinergic neurons. PHA-L was iontophoretically delivered to different hypothalamic and brainstem regions known to project to the rostral forebrain, and sections processed for the simultaneous detection of PHA-L labeled fibers/terminals and CBP-containing neurons using nickel enhanced DAB/DAB double labeling technique at the LM level. CaBP neurons in the lateral septal nucleus appear to receive contacts from the anterior hypothalamic nucleus, lateral hypothalamus, and locus coeruleus. PV containing neurons in the vertical and horizontal limbs of the diagonal band nuclei and lateral preoptic area are likely to be contacted by fibers originating from pontine tegmental regions and the ventral medullary reticular formation. Supported by USPHS Grant NS. 23945 and 17743.

398.14

IMMUNOCYTOCHEMICAL DEMONSTRATION OF GAMMA-AMINOBUTYRIC ACID IN ASTROCYTES LOCATED IN WHITE MATTER. M.S.Bull and A. Blomqvist, Dept. Cell Biol., Univ. Hospital, 581 85 Linköping, Sweden.

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

Adult rats were injected with gamma-acetylenic GABA (GAG)(i.p.200 mg/kg), a GABA-transaminase inhibitor, and subsequently perfused with a 1% paraformaldehyde-2% glutaraldehyde solution. Vibratome sections were cut through the brain and spinal cord and processed for preembedding light and electron microscopic GABA immunocytochemistry. Light microscopic examination revealed immunoreactive neurons but also numerous smaller immunoreactive profiles in the corpus callosum, diencephalic fiber tracts and nuclei, and spinal cord, which in the electron microscope were identified as astrocytes.

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

CATECHOLAMINES II

399.1

OSCILLATION OF INTERSPIKE INTERVAL LENGTH IN CENTRAL MONOAMINERGIC NEURONS. J.H. Carlson, C.W. Berridge and S.L. Foote. Dept. Psychiatry, UCSD, La Jolla, CA 92093.

The spontaneous discharge activity of individual monoamine-containing neurons was studied in halothane-anesthetized rats using extracellular recording techniques. Three measures were utilized: 1) mean discharge rates, 2) population characteristics of interspike interval (ISI) samples, and 3) ISI time series measures to examine patterns in the ordering of ISIs. The mean discharge rates of dopaminergic (DA) substantia nigra pars compacta and ventral tegmental area neurons and noradrenergic locus coeruleus neurons were 2.9±0.3 (n=16), 4.6±0.5 (n=12) and 1.4±0.2 (n=8) Hz, respectively. The ISI histograms for all 3 cell populations were similar to those described by others. Additionally, the time series analyses revealed a conspicuous oscillatory tendency in the sequence of ISIs in these neurons: Consecutive ISIs tended to alternate between short and long durations, although short bursts were evident in the DA cells. For all 3 cell types, long-short and short-long pairs of consecutive ISIs occurred significantly more frequently ($P<0.01$) than in the same samples when the order of ISIs was randomized. These oscillatory patterns are presumably produced by the operation of ionic conductances which terminate bursting activity, decreasing the probability of consecutive short ISIs, as well as conductances which serve to minimize the probability of consecutive long ISIs. The present analyses provide quantitative measures of the impact of these (and other) conductances on discharge activity.

399.2

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

The functional properties of midbrain dopaminergic (DA) neurons change following a loss of cells in the nucleus. The purpose of the present study was to determine whether the increase in dopamine turnover that results from DA cell loss is related, in part, to changes in DA neuronal impulse flow. Groups of BALB/cJ mice were treated with 40, 50 and 55 mg/kg 2'CH3-MPTP. Standard in vitro electrophysiological recording and HPLC procedures were used. The baseline firing rates of nucleus A9 neurons increased significantly (78%) as forebrain dopamine levels decreased. Autoreceptor-induced inhibition in cell firing (200 µM dopamine) was increased 40% in nucleus A10 but decreased in nucleus A9. There was also a 3 fold increase in cell incidence within nucleus A9 in the treated mice. These data suggest that as midbrain DA cell number decreases there is an increase both in the number of spontaneously active cells and in the firing rates of the active cells. Research supported by NIMH (MH-30546).

399.3

EFFECTS OF APAMIN ON THE ELECTROPHYSIOLOGICAL RESPONSE PROPERTIES OF MIDBRAIN DOPAMINERGIC NEURONS IN VITRO. D.C. German, X. Gu* and A. L. Blatz* . Depts. of Physiol. and Psychiat., U. of Texas Southwestern Med. Cntr., Dallas, TX 75235.

Midbrain dopaminergic (DA) neurons in vivo exhibit either a regular, irregular or burst firing pattern. In vitro, however, these neurons characteristically fire with a non-burst pattern. The purpose of the present experiment was to determine whether apamin, which blocks calcium-activated potassium channels, influences the activity of all midbrain DA neurons. Mice (20-25 g) were studied with standard in vitro extracellular single unit recording procedures. Cells were tested with superfused apamin (1µM) and dopamine (10-200 µM). Apamin often caused cells to change from a regular to irregular or burst firing pattern (n = 6) and caused some cells to slow or stop firing altogether (n = 5). Two regular-firing cells increased in rate but did not change in pattern. Apamin did not block the inhibitory effects of dopamine on any cell (n = 7). The variability in response to apamin is consistent with the hypothesis that not all midbrain DA neurons possess calcium-activated potassium channels. Research supported by NIMH (MH-30546) and NIH (GM-39731).

399.4

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

The effects of acute administration of RIS, a D2, S2 antagonist on DA neurons has been described. Little is known, however, about the chronic effects of RIS on the activity of DA neurons. In preparation for a study of chronic effects, the percent of midbrain DA neurons in the SNC that are active one hour after RIS was estimated by sampling the number of active DA neurons in multiple tracts through the midbrain (cell/tract sampling technique). The effects of RIS (0.5 mg/kg, i.p.) and the D2 antagonist HAL (0.5 mg/kg, i.p.) were compared with effects of saline. All drugs were administered one hour before the initial dose of anesthesia (chloral hydrate), and recording were begun about one-half hour later. In each experiment, 9-12 electrode tracts were made in the (SNC). DA neurons were identified using standard criteria. One hour following saline administration there was an average of 1.1 active DA cells per tract (C/T) in the SNC (n=7). HAL produced a 36% increase in active DA C/T in the SNC (n=9). This observation is consistent with other studies that have shown neuroleptics to increase the number of active DA neurons. RIS, on the other hand, produced a 27% decrease in the active DA C/T in the SNC (n=8). There were no differences in the mean discharge frequency of DA neurons in any treatment group. These results suggest that RIS is not typical of other neuroleptics since it does not increase the number of active DA C/T following i.p. administration.

399.5

EFFECT OF HALOPERIDOL (HAL) AND RISPERIDONE (RIS) ON DOPAMINERGIC (DA) NEURONS: CHANGE IN SPONTANEOUS ACTIVITY AND REVERSAL OF APOMORPHINE (APO)-INDUCED INHIBITION. P.S. Blum and D.M. Robisch, Dept. of Biol. Res., Janssen Res. Found., Spring House, PA 19477.

The antipsychotics RIS, an S-2, D-2 antagonist, and HAL, a selective D-2 antagonist, were administered intravenously to chloral hydrate anesthetized rats while recording from midbrain dopaminergic DA neurons using standard techniques. Fifty-five DA neurons were tested for altered spontaneous activity. Neurons with slow (<4.0 Hz) and rapid (<4.0 Hz) spontaneous activity were analyzed separately. HAL increased the activity of slow neurons (n=7; 165% of initial activity at 1.27 mg/kg) but did not affect rapid neurons (n=11; 108% of initial activity at 1.27 mg/kg). In contrast, RIS did not affect slow neurons (n=5; 100% of initial activity at 1.27 mg/kg), but decreased the activity of rapid neurons (n=10; 85% of initial at 1.27 mg/kg). The spontaneous activity of 18 other RIS-treated neurons (55% of total) ceased completely compared with 4 HAL-treated neurons (18%). In a previous study (Soc. Neurosci. Abstr., 14:1213, 1988), RIS was unable to block inhibition of DA neurons produced by APO, but in this study, intravenous RIS and HAL reversed APO-induced inhibition of activity in 34 additional DA neurons. The mean dose of HAL to produce a 50% reversal of neural activity (ED50) was 57 μg/kg. This value was about 2-3 times less than the mean ED50 for RIS (184 μg/kg). These data show that RIS and HAL can reverse the effect of a D-2 agonist on DA neurons, but they have a different effect on spontaneous activity of DA neurons.

399.6

D1 RECEPTOR STIMULATION INHIBITS THE ACTIVATION OF TUBEROINFUNDIBULAR DOPAMINE NEURONS INDUCED BY NEUROTENSIN, RESERPINE OR HYPERPROLACTINEMIA. S.A. Berry*, H.Y. Meltzer and G.A. Gudelsky. Departments of Psychiatry and Pharmacology, Case Western Reserve University, Cleveland, Ohio 44106.

In the present study we sought to determine the effects of D1 receptor activation on the activity of tuberoinfundibular dopaminergic (TIDA) neurons, as estimated from the accumulation of DOPA in the median eminence after decarboxylase inhibition. Treatment with the D1 agonist SKF 38393 (5-20 mg/kg) or CY 208-243 (10 mg/kg) alone did not alter the activity of TIDA neurons in male rats. However, SKF 38393 and CY 208-243 did inhibit the activation of TIDA neurons elicited by the injection of reserpine or neurotensin or by the induction of hyperprolactinemia with haloperidol. It is concluded that D1 receptor activation results in no effect on the basal activity of TIDA neurons, but that under conditions in which the activity of these neurons has been increased, D1 receptor activation elicits a marked inhibition of these neurons.

399.7

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

ECS is effective in treating various psychiatric illnesses including depression, in whose aetiology both DA and NA have been implicated. In addition, ECS has been shown biochemically and behaviourally to affect both DA and NA systems. We investigated neurophysiological changes induced in DA and NA neurones by ECS. Unanaesthetised rats received either repeated (one every second day for 10 days) or a single treatment (each being 800 ms of 120 V, 50 Hz stimulation). Sham rats received zero V. 24 h after the last treatment, spontaneous single cell discharge was examined under chloral hydrate anaesthesia in either the ventral tegmental area (VTA) or locus coeruleus (LC). VTA DA cell firing was more regular following repeated ECS than in sham rats, evidenced by a reduced variation coefficient (VC $P<0.01$). This reduced VC was significant for bursting ($P<0.02$) but not for non-bursting ($P>0.05$) cells. There were no significant differences in firing rate or the amount of burst firing after ECS. VTA cell discharge did not differ significantly after a single ECS treatment, although there was a trend for the VC to be lower in the ECS than the sham group ($0.05<P<0.1$). There were also no significant differences for VC, firing rate or burst firing in the NA cells of the LC after repeated ECS. These results show that the firing pattern of VTA DA cells is altered by repeated ECS treatment, in the absence of any significant change in NA cells of the LC. This change in firing pattern may have relevance for the use of ECS in treating, e.g., depression. #, Permanent address, Dept. Biological Sciences, Cumberland College of Health Sciences, Sydney, Australia

399.8

EFFECTS OF QUINPIROLE AND CHOLECYSTOKININ OCTAPEPTIDE ON DOPAMINE NEURONAL ACTIVITY IN THE MIDBRAIN SLICE. A.S. Freeman, D.J. Henry and L.A. Chiodo. Laboratory of Neurophysiology, Center for Cell Biology, Sinai Research Institute and the Cellular & Clinical Neurobiology Prog., Wayne State University, Detroit, MI 48235.

Dopamine (DA) and sulfated cholecystokinin octapeptide (CCK) coexist in a subpopulation of midbrain neurons and have been shown to influence the pre- and postsynaptic effects of one another. We report here preliminary data on the effcts of the D2 agonist quinpirole, CCK and the CCK antagonists proglumide and CR 1409 on the firing rate of DA cells in the in vitro superfused rat brain slice (450 um). Extracellular recording techniques were employed. Solutions of drugs were prepared in artificial CSF and sequentially delivered via a 4-way valve (2 ml/min). Quinpirole (5nM-1uM, n=7-15 cells/dose) produced dose-related inhibition of the spontaneous discharge of substantia nigra zona compacta (A9) DA cells (ED50 app. 40nM). CCK (0.1-100nM) excited most ventral tegmental area (A10) and medial A9 cells tested (29/40). Proglumide (10uM-10mM, n=13) generally had no effect on A10 and medial A9 cells although a few cells were inhibited. CR 1409 (10nM-1uM, n=12) exerted erratic effects on the firing rates of a similar population of cells but inhibitory responses predominated. Interactions among these compounds are under investigation. Supported by MH42136, MH41557 and Sinai Research Institute.

399.9

THE ROLE OF D1 AND D2 DOPAMINE RECEPTORS IN REGULATING THE ELECTROPHYSIOLOGICAL ACTIVITY OF CAUDATE NEURONS IN THE RAT. R. Shen, A.S. Freeman, D. Asdourian and L.A. Chiodo. Laboratory of Neurophysiology, Center for Cell Biology, Sinai Research Institute, the Cellular and Clinical Neurobiology Program and the Department of Psychology, Wayne State University, Detroit, MI 48235.

Extracellular single-unit recording and iontophoretic techniques were utilized to examine the effects of the selective D1 and D2 dopamine (DA) receptor agonists SKF 38393 and quinpirole on Type I caudate (Cd) neurons in paralyzed and chloral hydrate anesthetized rats.

SKF 38393 inhibited the basal firing rate of Type I Cd neurons in a dose-dependent manner in both alpha-methyl-para-tyrosine pretreated and normal rats, an effect blocked by the D1 DA antagonist SCH 23390. Quinpirole at ejection currents 10-40 nA exerted excitatory, inhibitory, or no effect on the basal firing rate of these neurons. A higher ejection current (80nA) inhibited the basal firing rate of most of the Type I Cd neurons, an effect blocked by the D2 DA antagonists sulpiride and eticlopride.

To date, no synergism between D1 and D2 agonists has been observed.

(Supported by MH41557 [LAC], MH42136 [ASF], and the Sinai Res. Inst.)

399.10

EFFECTS OF (+)-4-PROPYL-9-HYDROXYNAPHTHOXAZINE [(+)-PHNO] ON MIDBRAIN DOPAMINE NEURONS: AN ELECTROPHYSIOLOGICAL STUDY. M.D. Kelland, A.S. Freeman and L.A. Chiodo. Lab. of Neurophysiology, Center for Cell Biology, Sinai Research Institute and the Cell. and Clin. Neurobiology Program, Wayne State University, Detroit, MI 48235.

Standard extracellular single-unit and iontophoretic recording techniques were utilized to examine the effects of the D2 DA receptor agonist (+)-PHNO on identified nigrostriatal (NSDA) and mesoaccumbens (MADA) DA neurons.

(+)-PHNO administered i.v. inhibits the basal firing rate of NSDA neurons in a rate-dependent manner in both chloral hydrate-anesthetized and paralyzed rats. Significantly higher doses were required in the paralyzed preparation. Similar effects were observed with regard to the MADA neurons, with the exception that the inhibition was not rate-dependent in the paralyzed rats. (+)-PHNO administered iontophoretically also inhibited the firing rate of both NSDA and MADA neurons.

Neither SKF 38393 pretreatment nor hemitransection of the forebrain altered the rate-dependent responsiveness of NSDA neurons to (+)-PHNO. Likewise, depletion of brain 5-HT with either PCPA or 5,7-DHT failed to alter the rate-dependent responsiveness of these cells to (+)-PHNO. Thus, the ability of (+)-PHNO to inhibit NSDA neurons does not appear to be influenced by afferents.

(MH41557 [LAC], MH42136 [ASF], Sinai Res. Inst.; MDK is a Tourette Syndrome Association Postdoctoral Fellow)

399.11

REPEATED AMPHETAMINE ADMINISTRATION: RESPONSES OF NIGROSTRIATAL DOPAMINE NEURONS TO DOPAMINE AGONISTS.
D.K. Pitts, M.D. Kelland, A.S Freeman and L.A. Chiodo. Lab. of Neurophysiology, Center for Cell Biology, Sinai Research Institute and the Cell. & Clin. Neurobiology Program, Wayne State University, Detroit, MI 48235.

A previous study (Pitts et al., E.J.P. '89) examined the responses (change in discharge rate) of nigrostriatal dopamine neurons (NSDA) to i.v. apomorphine (APO) or quinpirole (QUIN) following repeated d-amphetamine (DAMP) treatment (4mg/kg/day i.p., 14 days). In that study NSDA neurons exhibited reduced sensitvity to APO but not to QUIN. Experiments utilizing SKF 38393 and SCH 23390 indicated that D1 receptor activation was important for the expression of reduced APO sensitivity. The effects of this DAMP regimen on NSDA neurons has recently been studied in experiments utilizing rats with a hemitransection of the midbrain/forebrain or in iontophoretic experiments examining the responsiveness of somatodendritic autoreceptors to dopamine in intact rats. Results from these experiments suggest that the D1 receptors which are responsible for the shift in the APO dose-response curve are located in the forebrain. A higher daily dose of DAMP (6 mg/kg/day, 14 days) decreased the sensitivity of NSDA neurons to quinpirole. Supported by MH41557 (LAC), MH42136 (ASF), MH09781 (DKP) and the Sinai Research Institute. MDK is a Tourette Syndrome Association Fellow.

399.12

WHOLE-CELL VOLTAGE-CLAMP ANALYSIS OF K CURRENTS IN ACUTELY DISSOCIATED, IDENTIFIED NIGROSTRIATAL DOPAMINE NEURONS.
L.A. CHIODO. Lab. Neurophysiology, Ctr. Cell Biol., Sinai Res. Inst. and Cell. and Clin. Neurobiol. Prog., Wayne State Univ. Sch. Med., Detroit, MI 48235.

We have been able to isolate identified, nigrostriatal dopamine (DA)-containing neurons from the mesencephalon of adult rats after retrograde labeling of their soma with rhodamine-conjugated latex microspheres (injected into the head of the caudate nucleus a minimum of 24 hrs earlier). These neurons have been studied electrophysiologically using whole-cell patch recording methods. The observed resting membrane potentials ranged between -53 to -64 mV, input resistances were 150-370 megohms and these cells had Na-dependent somatic action potentials (blocked by 1-10 uM tetrodotoxin). Voltage-clamp analysis, from a holding potential of -55 mV and after the removal of Na currents, revealed a late-onset, non-inactivating outward current in response to depolarization. This current was eliminated by the replacement of K ions in the patch electrode with Cs indicating that they were K-dependent. This K current could be separated into at least two components I_K (TEA-sensitive) and $I_{K(Ca)}$ (TEA-insensitive and blocked by Cd) with the latter accounting for 30-50% of the overall K current. (This work was supported by NIMH grant MH-41557 and the Sinai Res. Inst.)

399.13

DOPAMINERGIC NIGRO-STRIATAL NEURONS: EFFECT OF SKF 77434 ON FIRING RATE.
M. Diana, C. Okuda and P.M. Groves. University of California San Diego, La Jolla CA 92093.

Previous studies have indicated that stimulation of dopamine D1 receptors, either by peripheral injection or iontophoretic application in the substantia nigra does not affect the firing rate of dopaminergic neurons. However, virtually the totality of these studies have employed as a selective agonist the benzazepine SKF 38393. Recently SKF 77434, a N-allyl substituted benzazepine, has become available and classified as a D1 selective agonist on the basis of in vitro binding studies. We tested the possibility that this compound may influence dopaminergic neurotransmission by altering the firing rate of antidromically identified nigro-striatal dopaminergic neurons. Male Sprague-Dawley rats were anesthetized with urethane and placed in a stereotaxic frame. Dopamine neurons were identified according to well established electrophysiological criteria. SKF 77434 was administered intravenously through a catheter implanted in the femoral vein at exponentially increasing doses at 120 second intervals (3.9-125 µg/kg i.v.). Average firing rate of the entire 120 second period was compared to the predrug baseline. SKF 77434 produces a dose-dependent inhibition of the firing rate of nigro-striatal neurons. In some case SCH 23390 (D1 antagonist) was administered following SKF 77434. A reversal of the SKF 77434-induced inhibition was observed. Experiments are under way to ascertain if the SKF 77434 induced inhibition is a pre or a post-synaptic effect.

399.14

CALCIUM CURRENTS RECORDED FROM NIGROSTRIATAL DOPAMINE NEURONS ACUTELY DISSOCIATED FROM THE POSTNATAL RAT.
N.L.Silva and J.L.Barker, Laboratory of Neurophysiology, NINDS/NIH, Bethesda, MD 20892

Nigrostriatal dopamine neurons play a vital role in motor function. We injected fluorescent rhodamine labelled microspheres into the caudate nucleus of the postnatal (7-8 day) rat to retrogradely label nigrostriatal neurons. Cells visualized with fluorescence microscopy corresponded 89% of the time to neurons in the substantia nigra zona compacta (SNZC) which were identified as catecholaminergic following processing with FAGLU. Following a 4-5 day survival period the SNZC was enzymatically dissociated for 1 hr. Numerous neurons labelled with the fluorescent microspheres were observed to have phase bright somas. Cells were used for voltage clamp recordings for 6-8 hrs.

Calcium currents were isolated using an extracellular bathing medium which consisted of (mM) TEACl 145, HEPES 10, BaCl 3-5, MgCl 1, glucose 6 and the intracelluar medium contained NMGCl 120, Hepes 5, BAPTACs 5, MgATP 5, Tris phosphocreatinine 20, creatinine kinase 20U/ml. Two distinct components of calcium current were distinguished by their voltage and inactivation kinetics. A transient current (τ=150 ms) was activated with voltage steps more depolarized than -30 mV and had an inactivation range between -40 an -110 mV. A second sustained current ($\tau \geq$1.5s) was activated with voltage steps more depolarized than -20mV from a holding potential of -40 mV. The sustained current was sensitive to dihydropyridines. Both currents were blocked by low concentrations of cadmium and ω-Conotoxin. These results suggest that these two currents are similar to the "L" and "N" type currents which were originally described by Fox, Nowycky and Tsien (J. Physiol. 394:149-172, 1987).

399.15

ELECTROPHYSIOLOGICAL EFFECTS OF SELECTIVE OPIATE RECEPTOR AGONISTS ON A10 DOPAMINE (DA) NEURONS.
M. Jeziorski and F. J. White, Neuropsychopharmacology Lab, Lafayette Clinic, Cellular and Clinical Neurobiology Program, Dept. of Psychiatry, Wayne State Univ. Sch. Med., Detroit, MI 48207.

The mesolimbic (A10) DA pathway has been implicated in the rewarding properties of opiates and other drugs of abuse. Mu and kappa opiate agonists have been shown to exert contrasting effects on several indices of DA cell functioning, including rewarding and aversive effects, respectively. The mu agonist morphine is known to increase the firing rate of A10 DA cells, but the effects of other opiate agonists on A10 DA cell electrophysiology are not well understood. Extracellular single unit recordings were conducted in chloral hydrate-anesthetized rats to investigate the ability of cumulative i.v. doses of the selective kappa opiate agonist U50488h to alter A10 DA cell firing. U50488h (total dose 8 mg/kg) inhibited the firing of 7 of 9 cells to an average of 54% of basal firing rate. One cell increased its firing to 137% of basal rate, while another was unaffected. The mu selective antagonist naloxone (NAL), adminstered i.v. at doses of .02-.16 mg/kg, reversed the inhibition caused by U50488h in a dose-dependent manner. However, NAL administered alone did not alter A10 cell firing in 3 animals tested. Future experiments will characterize the effects of the kappa antagonist binaltorphimine, as well as the effects of locally applied agonists and antagonists on A10 DA cell firing. (Supported by USPHS grants DA-04093 and MH-40832.)

399.16

CONTINUOUS ADMINISTRATION OF D1 DOPAMINE (DA) RECEPTOR ANTAGONISTS SELECTIVELY INACTIVATES A10 DA NEURONS IN THE RAT.
S.R. Wachtel and F.J. White. Wayne State Univ., Sch. of Med., Dept. Psychiatry, CCN Program, Neuropsychopharmacology Lab, Lafayette Clinic, Detroit, MI 48207

It has been proposed that D1 dopamine (DA) receptor antagonists may possess the characteristics of atypical antipsychotic drugs (APDs). The present experiments investigated the effects of the prototypical D1 antagonist SCH 23390 and the new D1 antagonist NO-112 [(+)-8-chloro-7-hydroxy-3-methyl-5(7-benzofuranyl)-2,3,4,5,tetrahydro-1H-3-benzazepin] on the activity of A9 and A10 DA neurons using an electrophysiological model predictive of the clinical profile of APDs. Following 28 days (0.29 mg/kg/day) of continuous administration of NO-112 or SCH 23390 via subcutaneous mini osmotic pumps, selective decreases, 28.9% and 28.2% respectively, in the number of spontaneously active A10 DA neurons were observed. This selective effect is similar to, though smaller than, that previously reported for the atypical APD clozapine. However, in contrast to other APDs, the decrease in the number of A10 DA cells/track did not appear to result from depolarization inactivation, since the DA agonist apomorphine (10 or 50 ug/kg, i.v.) did not reverse the effect of NO-112. These results suggest that D1 DA receptor antagonists may possess an atypical APD profile with respect to midbrain DA neurons that occurs via a mechanism other than depolarization inactivation. (Supported by MH-40832, DA-04093 and by NOVO Industri A/S.)

399.17

STIMULUS FREQUENCY-DEPENDENT DOPAMINE AUTORECEPTOR ACTIVITY INVESTIGATED IN VITRO USING RAT NIGROSTRIATAL "CORE" EXPLANTS AND MICROELECTRODE ELECTROCHEMISTRY. M.D. DAVIS* AND G.A. GERHARDT (SPON: W. MOOS). Parke-Davis Research Division, Ann Arbor, MI 48105, and Depts. of Psychiatry and Pharmacology, Univ. of Colorado, Denver, CO 80262.

Dopaminergic (DA) neurotransmission in the striatum is regulated in part via nerve terminal autoreceptors of the "D2" subtype. Autoreceptor agonists inhibit striatal DA release more efficiently during low frequency electrical stimulation, while autoreceptor antagonists facilitate release more effectively at higher frequencies. We studied the effects of DA autoreceptor agents on catecholamine (CA) overflow during short trains of stimuli using in vitro nigrostriatal "core" explants and electrochemical analysis.

Nigrostriatal explants containing the soma-to-terminal continuum were obtained from Sprague-Dawley rats and perifused in a flow-through tissue chamber. A stimulating electrode was placed in the MFB explant region while a nafion-coated 30um diameter carbon fiber recording electrode was inserted into the striatal region. Chronamperometry as well as fast-scan cyclic voltammetry were employed to detect rapid changes in CA overflow. Electrical stimulation of the MFB for one second evoked frequency-dependent transient (5-10sec) increases in putative DA overflow. Bath administration of apomorphine (100nM) attenuated stimulated overflow by a greater degree at 10 vs 20 Hz whereas haloperidol (50nM) administration elevated stimulus-evoked overflow greater at 20 vs 10 Hz.

399.18

PHARMACOLOGICAL PROTECTION AGAINST THE EFFECT OF REVERSIBLE HYPOFRONTALITY ON RAT MIDBRAIN DOPAMINE NEURONS IN VIVO. J. Grenhoff*, C.-S. Tung* and T.H. Svensson. Dept. of Pharmacology, Karolinska Institute, Stockholm, Sweden.

Clinical neurophysiological studies have demonstrated that low activity in prefrontal/ frontal cortex (PFC) is a relatively consistent feature of chronic schizophrenia with negative symptoms. To study the possible influence of hypofrontality on the mesolimbocortical dopamine (DA) system, we recorded the spontaneous activity of A10 DA neurons in the anesthetized male rat during PFC inactivation induced by application of dry ice to the skull overlying the PFC. Application of dry ice in the midline 2 mm anterior to bregma specifically affected A10 DA cell firing in a reversible way. PFC cooling regularized the firing and abolished burst firing of A10 cells, i.e. a pacemaker-like firing was seen. In contrast, A10 cell firing rate was unaffected. Ritanserin blocked the effect of PFC inactivation on all A10 neurons (10/10), while amperozide blocked the effect on 9/18 A10 neurons. The actions of ritanserin and amperozide are interesting in view of the reported therapeutic action of these drugs in chronic (negative, type II) schizophrenia, a disorder characterized by hypofrontality and poor response to traditional neuroleptic treatment.

399.19

ALTERED RESPONSIVENESS TO HALOPERIDOL FOLLOWING PARTIAL DOPAMINE DEPLETION IN RATS: BEHAVIORAL AND ELECTROPHYSIOLOGICAL ASPECTS. J.R. Hollerman and A.A. Grace *Departments of Behavioral Neuroscience & Psychiatry, Center for Neuroscience, University of Pittsburgh, Pittsburgh, PA 15260.*

Compensatory mechanisms within the nigrostriatal dopamine (DA) system enable it to recover functionality after extensive damage, as evidenced by the extent of DA loss required before the emergence of symptoms in Parkinson's disease. This can be modeled in rats by the intraventricular administration of 6-hydroxydopamine (6-HDA), which results in destruction of DA terminals and retrograde degeneration of nigral DA cell bodies. Previous studies showed that rats can receive striatal DA depletions of up to 90% without exhibiting overt motor deficits. Furthermore, rats with larger depletions, which initially result in motor deficits (i.e., akinesia and catalepsy), often recover and resume normal motor behavior within 2 to 4 weeks. Nonetheless, motor deficits can be uncovered in both cases by exposure to stressors or acute treatment with neuroleptics. In this study we examined the effects of haloperidol (HAL) on the activity of nigral DA neurons and motor behavior 4-6 weeks following administration of 6-HDA. In DA depleted rats, i.v. administration of as little as 0.1 mg/Kg HAL caused spontaneously active DA cells to increase their firing rate, display prominent burst firing activity and then cease firing. Activity could be reinstated by systemic administration of apomorphine or iontophoretic application of GABA, suggesting that the DA neurons had entered a state of depolarization block. Parallel behavioral studies showed that the same dose of HAL (i.p) could induce a profound akinetic and cataleptic state (as measured by open field and raised platform tests) in the DA depleted animals while having no effect on controls in these tests. Studies are underway to determine the time course for the development of this alteration in the behavioral and electrophysiological response to HAL. Supported by MH09660 & NS19608.

399.20

IN VIVO BIOCHEMICAL CORRELATES OF ACUTE DEPOLARIZATION INACTIVATION IN SUBSTANTIA NIGRA DOPAMINERGIC NEURONS. E.D. Abercrombie, J.R. Hollerman, and A.A. Grace. *Department of Behavioral Neuroscience and Center for Neuroscience, University of Pittsburgh, Pittsburgh, PA 15260*

Although the chronic administration of neuroleptics abolishes the electrophysiological activity of dopamine (DA) neurons by means of depolarization inactivation (DI), DA turnover in postsynaptic regions is relatively unaffected. In this study, we used an acute model of DI combined with in vivo microdialysis to monitor DA release under these conditions. In this model, DI can be induced in substantia nigra DA neurons by administering a single dose of haloperidol (HAL; 0.5 mg/kg) to rats previously treated with 6-HDA, provided the striatal tissue DA depletion exceeds ~85% (Hollerman and Grace, 1989). This dose of HAL produced no significant alteration in extracellular DA from the basal level of 21 pg/sample (20 ul, corrected for recovery of the dialysis probe) in striata of 6-HDA treated rats (mean tissue DA depletion=90%; n=4). In intact rats, however, this dose of HAL increased both the firing rate of DA neurons and striatal extracellular DA (from a basal level of 41 pg/sample to 78 pg/sample; n=5). GBL (500 mg/kg), a compound that inactivates DA neurons via hyperpolarization, decreased extracellular DA in striatum to non-detectable levels in both 6-HDA treated (mean tissue DA depletion=89%; n=2) and control (n=3) rats. These data suggest that inactivation of nigral DA neurons by depolarization, as opposed to hyperpolarization, is not accompanied by a decrease in striatal DA release. Thus, DI may remove regulatory influences operative at the DA cell body while permitting DA release regulated at the nerve terminal to persist. Possible implications of these observations for a model of schizophrenia are discussed in the accompanying presentation (Grace et al., this meeting). Supported by the National Alliance for Research on Schizophrenia and Depression, and USPHS grants NS19608, MH42217, and MH09660.

399.21

PHASIC VERSUS TONIC DOPAMINE RELEASE AND THE MODULATION OF DOPAMINE SYSTEM RESPONSIVITY: A HYPOTHESIS FOR THE ETIOLOGY OF SCHIZOPHRENIA. A.A. Grace, E.D. Abercrombie & M.J. Zigmond. *Depts. of Behavioral Neuroscience and Psychiatry, Univ. of Pittsburgh, Pittsburgh, PA 15260.*

The dopamine (DA) hypothesis of schizophrenia, in its simplest form, suggests that this disorder is caused by DA system hyperactivity. However, there are several experimental findings that are inconsistent with this model: 1) increased DA metabolites have not been observed in schizophrenics, 2) there may be increased numbers of D2 receptors in schizophrenics, rather than the decrease that would be expected with overstimulation, and 3) homeostatic influences should compensate for simple increases in DA levels, thus restoring the system to normality.

Our model of schizophrenia is based on the premise that DA release occurs via two processes: 1) a transient or *phasic* DA release controlled by stimulus-induced changes in DA neuron firing, and 2) a more sustained, "background" *tonic* DA release which determines steady-state DA levels in DA terminal areas and is regulated by prefrontal cortical afferents. Behaviorally relevant stimuli (e.g., sensory inputs or affective states) would cause short-term activation of the DA system by increasing DA cell firing, thereby triggering phasic DA release. In contrast, the tonic component of DA release would serve to regulate the intensity of the response to phasic DA release by setting the background level of DA receptor stimulation (both postsynaptic and autoreceptor) in these sites. In the schizophrenic, a prolonged decrease in prefrontal cortical activity would markedly reduce tonic DA release. Over time, this would result in homeostatic compensations (e.g., receptor up-regulation, decreased feedback inhibition of DA synthesis & release) that would cause subsequent phasic DA release to elicit abnormally large responses. This DA system hyperresponsivity could be attenuated by neuroleptic-induced depolarization block of DA cells, since in this state stimuli would be incapable of activating DA cell firing, thereby averting the phasic response (see Abercrombie et al, this meeting).

399.22

ALTERATIONS IN DOPAMINE CELL ACTIVITY INDUCED BY PARTIAL DOPAMINE DEPLETIONS: EVIDENCE FOR DEPOLARIZATION BLOCK IN REMAINING CELLS. M.L.Pucak and A.A.Grace. *Depts. of Behavioral Neuroscience and Psychiatry, Center for Neuroscience, University of Pittsburgh, Pittsburgh, PA 15260.*

After partial depletions of the nigrostriatal dopaminergic pathway, remaining dopamine (DA) neurons exhibit increased firing rates (Hollerman & Grace, 1986) and can be driven into depolarization block (DB) by acute administration of the DA blocker haloperidol (Hollerman & Grace, 1988). We have investigated whether remaining DA cells in rats with 6-hydroxydopamine-induced partial depletions of the nigrostriatal tract undergo a spontaneous transition into DB by examining the effect of low doses of the DA agonist apomorphine (APO; 20 or 50 ug/kg, i.v.) on the number of spontaneously firing DA cells. Several lines of evidence suggest that some remaining DA cells may be in a state of DB by 4-6 weeks post-lesion (>85% depletion of striatal DA): 1) After administration of APO, the number of cells per track increased from .04±.08 to .13±.13 (n=8, p<.05). 2) DA cell firing rates were not as low after administration of APO as would be predicted if they had been spontaneously firing at the time of APO administration; that is, the firing rates of cells post-APO were not depressed (pre-APO, 50.7±9.5 (n=2); post-20 ug/kg APO, 60.4±25.9 (n=4); post-50 ug/kg, 48.7±13.7 (n=4)). 3) In one depleted rat, a DA cell was encountered which exhibited firing properties similar to that of DA cells entering DB. 4) Spontaneous firing has been elicited from a quiescent DA cell in a depleted rat by administration of iontophoretic GABA. These results may be of relevance in the treatment of Parkinson's disease, as clinical research has suggested that low doses of DA agonists decrease the number and severity of "off" periods in Parkinsonian patients (Stibe et al, 1988). Supported by USPHS NS19608.

399.23

TIME COURSE OF THE EFFECTS OF KAINATE-INDUCED PEDUNCULOPONTINE TEGMENTAL NUCLEUS (PPN) LESION SUGGESTS DEVELOPMENT OF SUBSTANTIA NIGRA PARS COMPACTA DOPAMINE CELL DEPOLARIZATION INACTIVATION RESULTS FROM LOSS OF PPN FUNCTION. M. Beninato, H.S. Pan and J.R. Walters. NINDS, Bethesda, MD 20892.

Previous studies (1) have shown a significant decrease in the number of spontaneously active (SA) substantia nigra pars compacta (SNc) dopamine (DA) neurons 7 days following kainate lesion of the PPN. Current studies using apomorphine pretreatment and GABA iontophoresis show that the silent DA neurons appear to be in a state of depolarization inactivation. Using previously described population study techniques (1) in chloral hydrate anesthetized rats, the time course of the development of this effect was studied. At 1 hour post lesion, the number of SA DA cells/pass (0.32±0.04, n=8) was significantly reduced from controls (0.82±0.09, n=6). Apomorphine pretreatment (80µg/kg) reversed this effect (0.92±0.13 cells/ pass, n=6). This indicated that the silent DA neurons are in a state of depolarization inactivation, resembling the condition seen at 7 days (0.50±0.07 cells/pass, n=9). At 12 hours post lesion, however, the number of SA DA cells/pass rebounded to control levels (0.95±0.07, n=6). These results suggest the depolarization inactivations observed at 1 hour and 7 days are due to different mechanisms. While the depolarization seen at 1 hour may be the result of an initial kainate-induced excitation of the PPN, this effect on the SNc DA neurons is apparently not sustained. The depolarization inactivation observed at 7 days may be due instead to the destruction of the PPN and subsequent alterations of neuronal influences on the SNc DA system.

1. Beninato et al., Soc. Neurosci. Abstr., 14(1): 407, 1988.

399.24

THE EFFECT OF CHRONIC NICOTINE ON THE ELECTROPHYSIOLOGICAL RESPONSE OF A9 AND A10 NEURONS TO SYSTEMIC NICOTINE. K.-W. Yoon*, M.J. Curfman* and T.C. Westfall (SPON: K. Smith). Depts. of Pharmacology and Neurosurgery, St. Louis Univ. Sch. of Medicine, St. Louis, MO 63104.

We have previously observed that the systemic administration of nicotine (NIC) increases the firing rate of midbrain dopamine (DA) neurons (Eur. J. Pharmacol. 141:395, 1987). In the present studies, the effect of i.v. NIC on the firing rates of A9 and A10 DA neurons in naive or rats chronically treated with NIC (s.c. implants for 7 or 14 days 1 mg/kg/day) was studied by extracellular single unit recordings. I.V. NIC increased the firing rate of both A9 and A10 cells in naive rats and as previously observed by us, NIC was more potent and efficacious in activating A10 than A9 neurons. Implantation of the NIC pellets had no significant effect on the basal firing rate of A9 cells while the basal firing rate of A10 cells was decreased from 3.3 to 2.1 and 2.6 Hz after 7 and 14 days, respectively. Chronic treatment of NIC for 7 or 14 days did not alter the increase in the firing rate of i.v. NIC in A9 cells. In contrast, chronic treatment with NIC produced a significant shift of the NIC i.v. dose response curve in A10 cells. The maximum change to NIC went from an increase of 84% in naive rats to 352% in rats chronically exposed to NIC for 14 days. It is concluded that systemic NIC preferentially stimulates A10 compared to A9 cells and this difference is augmented further by chronic NIC exposure. (Supported by DA 02688.)

SECOND MESSENGERS: CALCIUM

400.1

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

Using the intracellular fluorescent calcium-binding dye Indo-1 in conjunction with a fluorescence activated cell sorter, stimulation of 1321N1 human astrocytoma cells by muscarinic agonists was shown to result in a biphasic increase in the concentration of intracellular calcium of suspended cells. At least one component of this response is dependent on the presence of extracellular calcium. This component is not voltage dependent, and can be blocked by extracellular cobalt but not cadmium. Furthermore, this extracellular calcium-dependent component is not enhanced by higher concentrations of extracellular calcium and is not altered by the removal of extracellular sodium. Modeling of the kinetic components of the muscarinic calcium mobilization response in these cells suggest the involvement of at least three components which might be tenatively described as; a transient intracellular calcium release and resequestration of calcium that is not dependent on an influx of extracellular calcium, a more prolonged entry of extracellular calcium, and an efflux or further resequestration of cytosolic calcium.

400.2

GTP-γ-S DIFFERENTIALLY REDUCES CALCIUM CURRENT COMPONENTS IN RAT NODOSE GANGLION NEURONS. J.W. Wiley#, R.A. Gross, T.R.-Jastrow, R.L. Macdonald. Depts. of Neurology and #Internal Medicine, U of Michigan, Ann Arbor, MI 48104

Rat nodose ganglion neurons have 3 calcium current components similar to T, L and N currents in other sensory neurons. Neuropeptide Y selectively reduced the N current component via a pertussis toxin (PTX)-sensitive mechanism (Soc. Neurosci. Abstr. 14(1):645, 1988), suggesting that an inhibitory GTP binding (G) protein (G_i/G_o) couples certain receptors to voltage-gated calcium channels. We examined the effect of GTP and its stable thiol derivatives GDP-β-S (inactive ligand) and GTP-γ-S (activates G proteins) on the 3 calcium current components of rat nodose ganglion neurons. Currents were recorded from acutely-dissociated neurons from 7-10 d rats using the whole-cell variation of the patch clamp technique. The bath medium contained (mM): 10 HEPES, 67 choline, 100 TEA, 5.6 glucose, 5.3 KCl, 0.8 $MgCl_2$, 5 $CaCl_2$ (pH 7.4); the recording pipette (1-2.5 MΩ) contained 10 HEPES, 140 CsCl, 10 EGTA, 5 ATPMg and either 0.1 GTP, GDP-β-S, or GTP-γ-S. In control neurons (GTP), N and L current components decreased during the 20 min recording period. Substitution of GDP-β-S for GTP did not affect the amplitude, time to peak current, rate of inactivation, voltage ranges of activation or inactivation, or rate of "run-down" of calcium current components compared to controls. In contrast, GTP-γ-S differentially reduced the magnitude of calcium current components N>T>>L and increased the time to peak of N/L currents 3-4 fold. Pretreatment with PTX reversed the effects of GTP-γ-S. Thus, GTP-γ-S differentially affected the calcium current components in nodose ganglion neurons via a G-protein of the G_i/G_o type. Supported by VA Research Award to JWW, NIH 01019 to RAG, DA05345 to TR-J and DA04122 to RLM.

400.3

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

Acutely-dissociated rat nodose ganglion neurons have 3 calcium current components, similar to T, N and L currents of other sensory neurons. We used the purified catalytic subunit of protein kinase A (PKA) or the ATP analog ATP-γ-S, included in the recording pipette, to determine if kinase-mediated phosphorylation affected calcium currents in these neurons. In other preparations, cyclic AMP or PKA increases or removes inactivation from L-type currents (eg, Armstrong and Eckert, PNAS 84: 2518-2522, 1987). ATP-γ-S can serve as an irreversible phosphate donor in kinase reactions; we therefore compared its effect to that of PKA.

We used the whole cell variation of the patch clamp technique to record calcium currents from acutely-dissociated nodose ganglion neurons from 7-10 d rats. The bath medium contained (mM): 10 Hepes, 67 choline, 100 TEA, 5.6 glucose, 5.3 KCl, 0.8 $MgCl_2$, 5 $CaCl_2$ (pH 7.3); the recording pipette (1-2.5 MΩ) contained 10 Hepes, 140 CsCl, 10 EGTA, 5 ATPMg, 0.1 GTP. In control neurons, N and L current components declined throughout the recording period. PKA prevented "run-down" of the L current, evoked from holding potentials (V_h) = -40 mV, and enhanced the N current component (in currents evoked from V_h = -80 mV) while increasing its inactivation rate. ATP-γ-S had different effects, reducing calcium current magnitude and slowing the rate of current activation. These results suggest that PKA regulates both N and L current components. The effect of ATP-γ-S on calcium currents cannot be due to its ability to act as an irreversible phosphate donor via PKA.

Supported by NIH 01019 (RAG) and DA04122 (RLM).

400.4

INHIBITORS OF CALMODULIN AND PROTEIN KINASE C INFLUENCE INTRASYNAPTOSOMAL CALCIUM LEVEL AND TRANSMITTER RELEASE. M.H. Makman*, S.L. Garber and B. Dvorkin* (SPON: M.M. Rapport). Albert Einstein Col. of Med., Bronx, NY 10461

The Ca^{2+}-sensitive fluorescent dye, Fura-2, has been utilized to investigate regulation of internal [Ca^{2+}] in synaptosomes from specific regions of rat CNS including striatum. In striatal synaptosomes first equilibrated at 37° for 60 min and then loaded with Fura-2 acetoxymethyl ester, basal [Ca^{2+}] was stable at about 115 nM; following depolarization induced by 50mM K^+, within seconds [Ca^{2+}] increased to a new level of about 370 nM. Addition of 10µM 1-(5-isoquinolinyl-sulfonyl)-2-methyl piperazine (H7), an inhibitor of protein kinase C, caused a small but significant decrease (-20%) in basal [Ca^{2+}] and a major attenuation (-40%) of the effect of K^+-depolarization. The calmodulin inhibitor, N-(6-aminohexyl)-5-chloro-1-naphthalene sulfonamide (W7) at 10µM depressed basal [Ca^{2+}] slightly and almost completely aborted the K^+-induced increase in [Ca^{2+}]. The calmodulin inhibitor, calmidazolium (CMZ), at 10µM, unexpectedly increased [Ca^{2+}] to several-fold basal level, probably due to a calmodulin-independent effect. Correspondingly, CMZ, but not W7, caused release of transmitter (e.g. of -amino butyric acid and serotonin). H7 antagonized transmitter release. W7, H7 or CMZ did not alter striatal synaptosomal cyclic AMP. The effects of H7 and W7 support roles for calmodulin and protein kinase C in depolarization-induced [Ca^{2+}] influx and release of internal [Ca^{2+}].

400.5

REGULATION OF INTRACELLULAR CALCIUM BY CLONED MUSCARINIC ACETYLCHOLINE RECEPTOR SUBTYPES. J.D. Lechleiter, D.E. Clapham* and E.G. Peralta*. Dept. of Pharmacology., Mayo Clinic, Rochester, MN 55905 and Dept. of Molecular Biology, Genentech, Inc., San Francisco, CA 94143.

cDNA clones encoding human (H) muscarinic acetylcholine receptor (mAChR) subtypes HM1, HM2, HM3 and HM4 were introduced by the calcium phosphate method into chinese hamster ovary (CHO) cells lacking endogenous mAChRs. Stably transfected clonal cell lines were isolated as described previously (Peralta et. al. Sci. 236: 600-605, 1987). Single cell Ca^{2+} measurements were recorded using the Ca^{2+} dye indicators FLUO-3 AM (on a confocal microscope) and INDO-1 AM (on a flow cytometer). Acetylcholine (ACh)-induced increases in peak Ca^{2+} for flow cytometry are summarized below (concentrations are in nM).

	CONTROL		PTX TREATED	
TYPE	MAX / REST Ca^{2+}	ACh EC_{50}	MAX / REST Ca^{2+}	ACh EC_{50}
HM1	1985±32 / 45	177±11	1527±59 / 53	143±22
HM2	73±4 / 47	1070±220	no response / 47	---
HM3	194±5 / 72	14430±3600	65±10 / 88	9360±5950
HM4	2262±153 / 50	240±59	1838±89 / 64	111±23

The Ca^{2+} responses of HM1 and HM4 were the largest, the most sensitive to ACh and the least affected by PTX treatment (100ng/ml, 14-20 hrs). In contrast, the HM2 and HM3 responses were smaller and very sensitive to PTX. Chimeric mAChRs, stably transfected into CHO cells, are currently being examined. This work was supported by NIH (D.E.C.) and Genentech (E.G.P.)

400.6

PURIFICATION OF AN INOSITOL-1,4,5-TRISPHOSPHATE RECEPTOR FROM RAT SPLEEN. R.J. Mourey and S.H. Snyder. Johns Hopkins Univ. Sch. of Med., Dept. of Pharmacology and Molecular Sciences and Dept. of Neuroscience, Baltimore, MD 21205.

Inositol-1,4,5-trisphosphate (IP_3) binding sites in the rat have previously been described by our laboratory. In a screen of peripheral tissues, spleen and thymus displayed the highest source of IP_3 binding sites, while the cerebellum had by far the greatest binding in the CNS. Since spleen and thymus contain lymphocytes which, like neurons, are capable of intracellular communication, information transfer and long term memory, we decided to purify the IP_3 binding site from spleen and compare it to brain IP_3 receptors.

We have purified the spleen IP_3 receptor, which has similar high affinity and specificity for IP_3 as the brain IP_3 receptor. Interestingly, the spleen IP_3 receptor is smaller in molecular weight and does not cross-react antigenically to polyclonal serum made to the cerebellar IP_3 receptor. Antigenic cross-reactivity experiments with polyclonal serum made to spleen IP_3 receptor are underway. We have also shown that IP_3 potently releases calcium from both spleen and thymus microsomes, but not from microsomes preparted from tissue containing low amounts of IP_3 binding.

400.7

NEUROTRANSMITTER STIMULATED INCREASES IN MASS LEVELS OF INS(1,4,5)P_3 IN BRAIN SLICES AS MEASURED BY IP_3 RADIORECEPTOR ASSAY. D.S. Bredt, S.H. Snyder. Department of Pharmacology and Molecular Sciences and Department of Neuroscience Johns Hopkins Univ. Sch. of Med. Balt., MD 21205

The IP_3 receptor from crude rat cerebellar membranes was used to create a simple, sensitive, and specific radioreceptor assay for the determination of mass levels of IP_3 in biological tissues (Bredt, D.S., et al., BBRC, 159:976, 1989). The assay's detection limit is less than 1 pmole of IP_3, and it is specific for Ins(1,4,5)P_3 as demonstrated by both enzymatic and chromatographic studies. Using this assay we detect substantial increases in mass levels of IP_3 in in vitro slices of rat cerebellum in response to excitatory amino acids (EAA). Using 500 uM quisqualate, mass levels of IP_3 increase to 350% of baseline within 10 sec., decreasing to only 150% by 60 sec. The rank order of EAA's in terms of potency and efficacy is quisqualate > glutamate > kanate. These data demonstrate, for the first time, that neurotransmitters can stimulate large, transient increases in the mass levels of IP_3 in brain slices.

400.8

Ca^{2+}-DEPENDENT REGULATION OF INS-1,4,5-P_3 BINDING TO RAT CEREBELLAR RECEPTOR. S.K. Danoff, A. Theibert, R. Evans*, S. Snyder. Dept. of Neuroscience, Johns Hopkins Univ., School of Medicine, Baltimore, MD 21205

The Ins-1,4,5-P_3 (IP_3R) receptor purified from rat cerebellum (Suppatapone, S. et al, JBC, 263:1530, 1988) and recently functionally reconstituted (Ferris, C. et al, Neurosci. Abstr., 1989, in press) appears to act as an intracellular Ca^{2+} channel. Two distinct mechanisms of Ca^{2+} regulation have been demonstrated to function in this system. The first is a membrane-bound protein (calmedin) distinct from the IP_3R which mediates an EDTA reversible inhibition of binding with a Km for Ca^{2+} of 300nM.(Danoff, S. et al, Biochem. J., 254:701, 1988) This protein has been further purified and characterized.

A second mechanism is mediated by a membrane-bound, Ca^{2+}-dependent IP_3-phoshpatase. This protein has been partially purified and characterized. The phosphatase has a Km for IP_3 of ~10uM and an absolute dependence on Mg^{2+}.

400.9

CYTOSOLIC Ca^{++} TRANSIENTS IN BRADYKININ TREATED PC12 CELLS. F.Grohovaz[1,2], C.Fasolato[3], A Malgaroli[4] and G.Fumagalli[1,4] (SPON: L.Vicentini). [1]: CNR Ctr Cytopharmacol; [2]: Ctr Periph Neuropathies; [3]: Dept Pharmacol; [4]: San Raffaele Inst, Milano, Italy; [5]: CNR Ctr Biomembranes, Padova, Italy.

Recent studies on suspension of PC12 pheochromocytoma cells, loaded with fura-2, indicate that treatment with bradykinin (BK) elicits a redistribution of Ca^{++} from IP_3-sensitive stores and, in addition, activates Ca^{++} influx through voltage independent cationic channels (Fasolato et al. J. B. C. 263: 17350-59). From these studies it is not known whether the two effects on cytosolic Ca^{++} have a similar spatial localization or even whether all the cells display both these responses.

In order to addres these questions we have studied local $[Ca^{++}]_i$ changes by digital imaging of single PC12 cells loaded with fura-2. Cells exposed to BK (100 nM) in a Ca^{++}-free EGTA-containing medium showed rapid and uniform transient increases in $[Ca^{++}]_i$ occurring simultaneously over the whole cytoplasm. The reintroduction of Ca^{++}(2 mM) into the medium, gave way to sustained rises of $[Ca^{++}]_i$, mainly restricted to the periphery of the cell, whilst a subsequent addition of excess EGTA caused the rapid decline of $[Ca^{++}]_i$ in the same peripheral areas.

These data confirm the dual mechanisms of BK actions on $[Ca^{++}]_i$ and add the notion that IP_3 sensitive stores are evenly distributed throughout the PC12 cell cytoplasm.

400.10

STIMULATION OF CALCIUM INFLUX BY INOSITOLHEXAKISPHOSPHATE ($InsP_6$): POSSIBLE INVOLVEMENT OF A SPECIFIC CLASS OF MEMBRANE RECOGNITION SITES. V. Bruno*, S. Cavallaro*, M.A. Sortino, P.L. Canonico and F. Nicoletti* (SPON: M.J. Cronin). Dept. Pharmacology, University of Catania, Italy.

$InsP_6$ stimulates $^{45}Ca^{2+}$ uptake in cultured cerebellar neurons and anterior pituitary cells. The influx of extracellular Ca^{2+} is accompanied by translocation of protein kinase C and, in cerebellar neurons, by excitatory amino acid release. The action of $InsP_6$ is reproduced by inositolpentakisphosphate, but not by inositol-1,3,4,5-tetrakisphosphate ($InsP_4$). Low concentrations (5-100 uM) of divalent cations (Cu^{2+}, Mg^{2+}, Ni^{2+}, Co^{2+}) greatly enhance the stimulation of $^{45}Ca^{2+}$ influx by $InsP_6$. We have studied [^{3}H]$InsP_6$ binding in crude synaptic membranes from cerebral hemispheres or cultured cerebellar neurons, incubated at 37°C in Tris-HCl (pH 7.4). [^{3}H]$InsP_6$ binds to a single population of specific and saturable recognition sites, with a K_D value in the low nanomolar range. [^{3}H]$InsP_6$ binding is temperature-dependent, being virtually absent at 0°C. Specifically bound [^{3}H]$InsP_6$ is displaced by InsP6 and InsP4 but not by glutamate. We speculate that $InsP_6$ stimulates a specific class of membrane recognition sites, triggering the influx of extracellular Ca^{2+} in target cells.

400.11

PROLONGED PHORBOL ESTER PRETREATMENT DESENSITIZES THE κ OPIATE-INDUCED INHIBITION OF Ca^{2+} INFLUX IN RAT SPINAL CORD-DORSAL ROOT GANGLION COCULTURES. Z. Vogel, B. Attali*, D. Saya* and S.-Y. Nah*. Dept. of Neurobiology, Weizmann Institute of Science, 76100 Rehovot, Israel.

$^{45}Ca^{2+}$ uptake into rat spinal cord-dorsal root ganglion cocultures is stimulated by K^+ depolarization in the presence of Bay K8644, an agonist of the L type voltage-dependent Ca^{2+} channels. Opiate agonists of the κ type (e.g., U50488, tifluadom, ethylketocyclazocine, dynorphin) profoundly depress the K^+/Bay K-stimulated Ca^{2+} uptake (Attali et al., JBC, 264:347, 1989). Addition of the phorbol ester TPA (activator of protein kinase C) to the Ca^{2+} influx assay (5 min) decreased the K^+/Bay K-stimulated Ca^{2+} uptake ($IC_{50} \approx 70$ nM, 66% inhibition at 1 μM), but did not affect the inhibition produced by the opiates. However, prolonged preincubation (24 h) of the cells with 100 nM TPA (to allow down-regulation of protein kinase C) abolished the capacity of the κ opiatns, as well as of TPA itself, to inhibit the $^{45}Ca^{2+}$ influx through the voltage-dependent Ca^{2+} channels. A significant desensitization of the opiate effect was already observed following 1 h pretreatment with 100 nM TPA. This desensitization by TPA of the opiate inhibitory effect was dose-dependent with ED_{50} in the nanomolar range. The inactive phorbol ester (4β-phorbol 13 acetate) did not affect the opiate inhibition. These results suggest that protein kinase C affects Ca^{2+} channel activity and has a role in the modulation of Ca^{2+} channel activity by κ opiates.

400.12

LOW LEVELS OF INOSITOL PHOSPHATE FORMATION ARE ASSOCIATED WITH THE OPENING OF DIHYDROPYRIDINE-SENSITIVE Ca^{2+} CHANNELS BUT NOT Ca^{2+} MOBILIZATION IN BOVINE ADRENAL CHROMAFFIN CELLS. K.A. Stauderman, R.M. Pruss*, and M.M. Murawsky*, Merrell Dow Research Institute, 2110 E. Galbraith Road, Cincinnati, OH 45215

In single bovine adrenal chromaffin cells loaded with fura-2, histamine concentrations from 3-30 nM elicited slow, oscillatory increases of intracellular free calcium ($[Ca^{2+}]_i$). These responses were H_1-mediated, and were apparently due to Ca^{2+} entry through L-type channels as they were antagonized either by 0.1 μM mepyramine, 20 μM gadolinium (Gd^{3+}), omitting external Ca^{2+}, or by 1 μM (-)202,791, a dihydropyridine Ca^{2+} channel antagonist. In cell cultures, 3-30 nM histamine stimulated the accumulation of inositol 4- and 1-monophosphate (Ins(4)P and Ins(1)P), with Ins(1)P predominating.

Concentrations ≥ 100 nM histamine produced a rapid and relatively large transient of $[Ca^{2+}]_i$, often followed by a second transient of variable size, which then declined to a new plateau above prestimulation levels. Removal of external Ca^{2+}, 20 μM Gd^{3+}, or 1 μM (-)202,791 did not affect the initial transient, supporting a role for Ca^{2+} mobilization in this response. Interestingly, ≥ 100 nM histamine stimulated more Ins(4)P accumulation than Ins(1)P.

These data suggest that conditions causing preferential accumulation of Ins(1)P lead to activation of L-type Ca^{2+} channels, while Ca^{2+} mobilization occurs when conditions favor Ins(4)P accumulation.

400.13

BRADYKININ MODULATES POTASSIUM AND CALCIUM CURRENTS IN RAT PHEOCHROMOCYTOMA CELLS A. Villarroel*, N.V. Marrion* and P.R. Adams. (SPON: G. Matthews) Howard Hughes Medical Institute, Dpt. Neurobiol. SUNY SB, Stony Brook, NY 11794

PC12 cells fused with 50% polyethylene glycol and exposed to 50 ng/ml NGF for 2-8 days have been voltage-clamped in the whole-cell mode. The effects of bath- or puff-applied bradykinin (BDK) (1 μM) on ionic conductances were studied. BDK produced a membrane hyperpolarization mainly due to the development of an outward current (I_{BDKout}) that was blocked with 200 μM d-tubocurarine. The development of I_{BDKout} was accompanied by a two to threefold increase in $[Ca^{2+}]_i$, and was prevented when 10 mM BAPTA was included in the pipette (*cf.* Fasolato *et al.*, J.Biol.Chem. 263:17350, 1988). The hyperpolarization was followed by a depolarization attributable to inhibition of an M current (I_M). In contrast to NG108-15 cells (*cf.* Higashida H. & Brown D.A., Nature 323:333, 1986), neither bath-application of 1 μM Phorbol-12,13-Dibutyrate (PDBu), 250 μM L-α-Dioctanoyl-Glycerol, nor addition of 20 μM 1-Oleyl-2-Acetyl-Glycerol (OAG) in the electrode produced a reduction of I_M. (1,4,5)-IP_3 (up to 100 μM) or 10 mM BAPTA did not affect BDK-mediated inhibition of I_M when included in the recording pipette. After challenging with 10 mM caffeine, no effect on I_M and a negligible increase in $[Ca^{2+}]_i$ were detected.

Calcium currents were recorded in 0 K^+ and with CsCl and 1 mM EGTA in the electrode. Cells were held at -60 or -80 mV and jumped to 10 mV for 200 or 400 ms. Under these conditions, BDK produced a reversible reduction of the calcium current, without affecting significantly its decline during the pulse. In some cells a reduction of the holding current was observed. BDK's action on the voltage-dependent calcium current may be mediated by intracellular calcium, because it was suppressed by inclusion of 10 mM BAPTA in the pipette. PDBu (1 μM) produced a small increase in the initial inward current elicited by a jump to 10 mV, and a significant reduction of the current measured after 400 ms. This effect took several minutes to develop and was partially reversible.

400.14

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

The enzyme systems and processes mediated by the Ca^{2+}-binding protein, calmodulin (CaM), are thought to play an important role in stimulus-induced Ca^{2+} signalling. The effects of a muscarinic receptor-mediated Ca^{2+}flux on the activity and distribution of CaM was examined. We found that exposure of SK-N-SH cells to the muscarinic agonist carbachol results in a concentration and time-dependent increase in CaM activity in the cytosol. The EC_{50} for the response to carbachol is 1-2 μM, which correlates with the EC_{50} of carbachol for the phosphoinositide response of 10-30 μM, and the EC_{50} of 8 μM of the agonist oxotremorine-M for peak rise in intracellular Ca^{2+} ($[Ca^{2+}]_i$). The maximal increase in cytosolic CaM was with 10 μM carbachol from a control value of 2.9 ± 0.6 ng CaM/μg protein to 9.2 ± 2 ng CaM/μg protein. The increase in CaM activity for 100 μM carbachol occurred by 5 minutes and appeared to last for at least 30 minutes, with the maximal increase in the cytosol from 3.9 ± 0.3 ng CaM/μg protein to 10.7 ± 2 ng CaM/μg protein occurring at 15 minutes. There was no difference between the control cytosolic CaM levels and those measured after 2 hours of incubation with carbachol, and no difference in the CaM activity in the membrane fractions. The response to 10 μM carbachol was completely blocked by 1 μM atropine, and the response did not occur when the cells were stimulated with the nicotinic agonist dimethyl-4-phenyl piperazinium. These results suggest that the muscarinic receptor-mediated rise in $[Ca^{2+}]_i$ augments CaM activity in the cytosol of SK-N-SH cells. This increase in cytosolic CaM activity may represent the coupling of the $[Ca^{2+}]_i$ flux to enzyme activation.

SECOND MESSENGERS: PHOSPHOINOSITIDE TURNOVER

401.1

INCREASED $^{32}P_i$ INCORPORATION INTO BRAIN PHOSPHOINOSITIDES IN HEREDITARY MODEL OF EPILEPSY. J.M. Tuchek,* R.I. Wilcox,* D.D. Johnson and S.C.J. Pedder*. Dept. of Pharmacology, College of Medicine, Univ. of Sask., Saskatoon, Saskatchewan S7N OWO

Epileptic fowl are a consequence of an autosomal recessive mutation such that homozygous recessive birds have a low seizure threshold to a variety of stimuli including photic stimulation. Carriers (heterozygotes) have a normal seizure threshold. The rate of $^{32}P_i$ incorporation into phosphatidylinositol (PI), phosphatidylinositol-4-phosphate (PIP) and phosphatidylinositol 4,5 phosphate (PIP_2) was studied in synaptosomal preparations obtained from the forebrains of carrier and epileptic fowl. Synaptosomes were isolated in a standard ficoll/sucrose density gradient and resuspended in a buffer consisting of 110 mM NaCl, 5 mM KCl, 1.3 mM $MgCl_2$, 1.3 mM $CaCl_2$, 1 mM pyruvate, 10 mM glucose and 50 mM imidazole pH 7.4. An aliquot (1 mg Lowry protein) of this suspension was incubated at 37°C with 5 μCi ^{32} P_i (ICN) and buffer in a total reaction volume of 500 μl for periods up to 60 min. Labelling was terminated by the addition of a pre-mixed solution of $CHCl_3$/MeOH/2.4 N HCl (2/1/0.5). The aqueous layer was washed with $CHCl_3$ and the pooled $CHCl_3$ extracts were evaporated to dryness under N_2. The dried extract was resuspended in $CHCl_3$/MeOH (2:1) and the lipids were separated on silica gel 60 plates with $CHCL_3$/acetone/MeOH/acetic acid H_20 (80/30/26/24/16). PIP_2, PIP, PI and phosphatidic acid (PA) were identified with iodine vapor using appropriate standards. The lipid spots were scraped and the radioactivity was determined by liquid scintillation counting. The amount of $^{32}P_i$ incorporated into the phosphoinositol lipids and PA was approximately 35% higher in synaptosomes prepared from epileptic fowl than in the carrier birds. These data indicate that the low seizure threshold in epileptic fowl is associated with an increased basal turnover of the phosphatidylinositol system. Supported by the MRC.

401.2

STIMULATION OF INOSITOL PHOSPHOLIPID TURNOVER IN HUMAN RETINAL PIGMENT EPITHELIAL CELLS BY CARBAMOYLCHOLINE. E.L. Feldman, A.E. Randolph and D.A. Greene*. Depts. of Neurology and Internal Medicine, Univ. of Mich. Med. Ctr., Ann Arbor, MI 48109.

Human retinal pigment epithelial cells (HRPE) in culture demonstrate β-adrenergic activated adenylate cyclase activity (Friedman et al., Exp. Eye Res., 44:471, 1987). We report here the presence of a muscarinic cholinergic receptor in HRPE coupled to a different biochemical effector system, stimulation of phosphoinositide (PPI) turnover.

Cultured HRPE were incubated with ^{3}H-myo-inositol and treated with carbamoylcholine, resulting in a large accumulation of labeled inositol phosphates (>300% of control). Inositol phosphate accumulation was linear up to 60 min with an EC_{50} of 100 μM. Stimulation was calcium dependent (2 mM) and inhibited by atropine (10 μM). Sequential elution of inositol phosphates with ion exchange chromatography revealed that the major labeled component in stimulated HRPE was IP_1 (55% of total radioactivity), with smaller amounts of glycerophosphoinositol (12%), IP_2 (21%), and IP_3 (10%). In competition studies on HRPE, atropine (10 μM) inhibited a population of isolated ^{3}H-QNB binding sites. These results suggest the presence of a muscarinic receptor on HRPE linked to the PPI second messenger system.

401.3

STIMULATION OF INOSITOL PHOSPHATE METABOLISM BY EXCITATORY AMINO ACIDS IN NEONATAL RAT SPINAL CORD. M.N.Perkins*, K.Taylor* and G.M.Burgess*, (SPON: I.F.James). Sandoz Institute for Medical Research, Gower Place, London WC1E 6BN, UK.

Recent evidence suggests that excitatory amino acids (EAA's) activate polyphosphoinositidase C (PIC) in a number of neural tissues. As EAA's have been implicated in sensory neural transmission we have investigated the possiblity that EAA's activate PIC in spinal cord slices. Spinal cord slices from 1 day old rats were equilibrated with [^{3}H]-*myo*-inositol. The effect of EAA's (in the presence of atropine and lithium) on PIC activity was determined by measuring levels of [^{3}H]-inositol bisphosphate ([^{3}H]-IP$_2$). All EAA's tested caused an increase in [^{3}H]-IP$_2$ with EC$_{50}$ values of: glutamate (GLU) 500μM; kainate 40μM; NMDA 25μM; quisqualate 3μM. These responses were seen in the presence and absence of TTX. The response to NMDA was antagonised by MK801 (IC$_{50}$<500μM) and kynurenic acid (KYN IC$_{50}$<500μM) and by D(-)2-amino, 5-phosphopentanoic acid (AP5, IC$_{50}$ 60μM.). The response to quisqualate was not reduced by 500μM KYN, N-(p-bromobenzoyl)piperazine- 2,3-dicarboxylate (pBB-PzDA) or AP5. The response to kainate was reduced by 200μM pBB-PzDA but not by KYN. The response to GLU was not blocked by any of these antagonists, nor by L(+)2-amino-4-phosphonobutyric acid (500μM).

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

401.4

EFFECTS OF DOPAMINE AND CHOLECYSTOKININ ON PHOSPHATIDYL-INOSITOL TURNOVER - CA^{2+} SIGNALLING SYSTEM IN THE RAT STRIATUM. S. Kito, R. Miyoshi, A. Tanaka*, Y. Kimura* and T. Mitsuno*. Third Department of Internal Medicine, Hiroshima University School of Medicine, Hiroshima 734, Japan.

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

401.5

EFFECTS OF SIGMA COMPOUNDS ON AGONIST-STIMULATED PHOSPHOINOSITIDE METABOLISM IN RAT BRAIN. S.M. Candura*, T. Coccini*, L. Manzo[1] and L.G. Costa (SPON: J. Franck). Dept. of Environ. Health, Univ. of Washington, Seattle, WA and [1]Dept. of Pharmacology, Univ. of Pavia Medical School, Pavia, Italy.

Sigma receptors are non-opiod, non-dopaminergic receptors which are implicated in psychosis and in antipsychotic drug efficacy.The second messenger system coupled to these receptors is still unknown. Recently, an inhibitory effect of various sigma compounds on carbachol-stimulated phosphoinositide (PI) metabolism has been reported (Eur. J. Pharmacol. 149,399, 1988). We have investigated the effect of sigma compounds on carbachol - stimulated PI metabolism in rat cerebral cortex slices. (+) Pentazocine, 1,3,-di-o-tolylguanidine (DTG) and N-allylnormetazocine (SKF-10,047) inhibited carbachol-stimulated PI hydrolysis with IC$_{50}$s of 34, 53 and 230 μM, respectively. Haloperidol and 3-(hyproxyphenyl)-N-(1-propyl)-piperidine (3-PPP) caused a maximal 15-40% inhibition at 250-500 μM. In the presence of IC$_{50}$ concentrations of (+) pentazocine or DTG the dose-response curve for carbachol was shifted to the right and its EC$_{50}$ increased from 50 μM to 238 and 248 μM, respectively. Similar results were found in hippocampus and cerebellum. (+)Pentazocine, DTG and SKF-10,047 also inhibited the binding of ^{3}H-QNB to muscarinic receptors in cortical membranes with IC$_{50}$s of 21, 52 and 120 μM, respectively. These results suggest that sigma compounds can modulate muscarinic receptor-stimulated PI metabolism in brain tissue, possibly, at least in part, through an interaction with the muscarinic recognition site. (Supp. in part by grants from NIEHS, ES-04696, and Fondazione Clinica del Lavoro, Pavia).

401.6

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

We reported earlier that glutamate inhibited NE-stimulated PI hydrolysis in rat brain slices by inhibiting phospholipid synthesis through a quisqualate-selective site. We further found that sulfur-containing amino acids also inhibited NE-stimulated PI hydrolysis in rat brain slices. Of the tested sulfur-containing amino acids, L-cysteine was the most potent, inhibiting the NE-induced response by 42% and 85% at concentration of 50 μM and 500 μM, respectively. L-homocysteate slightly potentiated PI hydrolysis at a concentration of 100 μM, but it was inhibitory at 500 μM. The D-isomers of cysteine and homocysteate were much less potent than were the L-isomers. L-cysteine, L-cysteine sulfinic acid and L-serine-O-sulfate also inhibited PI hydrolysis, but the effect was moderate or mild compared to L-cysteine. Sulfur-containing amino acids did not cause cell lysis and their inhibitory effect was partially reversible. These results demonstrate that several sulfur-containing amino acids, some of which have been proposed to be endogenous excitatory amino acid neurotransmitters, effectively modulate the response of NE of the PI second messenger system in the rat brain. The possible mechanisms of the inhibitory effect caused by excitatory amino acids and sulfur-containing amino acids are being studied. It is possible that some calcium-regulated processes may be involved in the modulatory effects of these amino acids.

401.7

PCP INHIBITS CARBACHOL INDUCED PHOSPHOINOSITOL HYDROLYSIS IN THE RAT BRAIN. J.M. Brog and M.C. Beinfeld. Department of Pharmacology, St. Louis Univ. School of Medicine, St. Louis, MO 63104.

We have previously reported that while phorbol esters increased CCK release in various regions of the rat brain, phencyclidine had an inhibitory effect. In order to examine a possible relationship between inhibition of CCK release and the phosphoinositol cycle, we examined the effect of PCP on carbachol-induced phosphoinositol hydrolysis in rat brain slices from cortex, caudate-putamen, and the hippocampal formation. In all three regions studied, PCP significantly inhibited the carbachol-induced ^{3}H-myo-inositol-1-phosphate accumulation, exhibiting the greatest potency in the cortex at 10^{-6} M. Since PCP has been shown to have agonistic properties at sigma opiate receptors, its actions were compared to those of (+)SKF 10,047, a sigma opiate agonist. (+)SKF 10,047 inhibited the carbachol-induced phosphoinositol hydrolysis in similar concentrations to PCP in the three areas studied. It is thus quite possible that PCP is at least partially acting through the sigma opiate receptor to inhibit the carbachol effect. However, further studies will be required to elucidate its mechanism of action, and how this action of PCP may be related to its effect on CCK release. Supported by NIH NS18667.

401.8

ATP INDUCES POLYPHOSPHOINOSITIDE BREAKDOWN AND AN INCREASE IN CYTOPLASMIC, FREE CALCIUM LEVELS IN NEUROBLASTOMA X GLIOMA HYBRID CELLS.
T-A. Lin*, K.D. Lustig*, M.G. Sportiello*, G.Y. Sun and G.A. Weisman*. Department of Biochemistry and Sinclair Research Farm, University of Missouri-Columbia, Columbia, MO 65203

Extracellular ATP exhibits hormone-like properties in the peripheral and central nervous systems. In the present study, the addition of ≥ 1μM ATP to a neuroblastoma X glioma hybrid cell line (NG108-15) was found to induce a transient increase in the level of cytoplasmic, free calcium (Ca^{2+}_i). The increase in the Ca^{2+}_i level was maximal within ten second after addition of ATP and then declined to the initial level within one minute. However, the ATP-induced increase in the [Ca^{2+}_i] was greater in the presence than in the absence of extracellular calcium. Increases in the [Ca^{2+}_i] correlated with the concentration of the fully ionized form of ATP (ATP^{4-}) in the medium. Other nucleoside 5'-triphosphates, including UTP, ITP, TTP and GTP, also induced an increase in the [Ca^{2+}_i]. The addition of ATP to cells prelabeled with $^{32}P_i$ or [2-^{3}H]inositol resulted in a rapid decrease in the level of phosphatidylinositol 4,5-bisphosphate (PIP_2), the lipid precursor of inositol 1,4,5-trisphosphate (IP_3) and diacylglycerol (DG). ATP also caused an increase in the level of $^{32}P_i$-labeled phosphatidic acid, presumably as a result of phosphorylation of DG. These findings suggest that extracellular ATP^{4-} activates a signal transduction pathway in NG108-15 cells involving the formation of IP_3 and the mobilization of Ca^{2+}.

401.9

CHARACTERIZATION OF THE INOSITOL PHOSPHATE RESPONSE TO BRADYKININ IN NG108-15 NEUROBLASTOMA X GLIOMA HYBRID CELLS: EFFECTS OF LITHIUM. J.E. Rubinstein and R.J. Hitzemann. Dept. Psychiatry, SUNY: Stony Brook, NY 11794-8101.

We have been using the NG108-15 clonal cell line as a model to investigate the regulation of Phospholipase C-mediated phosphoinositide (PI) hydrolysis. NG108-15 cells were grown in DMEM with HAT and 10% FBS and labeled for 2 days with [^{3}H]inositol. The media was removed and the cells pre-incubated for 15 min in Ringer-HEPES with or without LiCl. Bradykinin (BK) was then added for various times and the reaction terminated with perchloric acid. Inositol phosphates were separated by ion-exchange chromatography.

BK stimulated PI hydrolysis in a dose-responsive fashion reaching a maximum at a concentration of 10-25 μM. The response was extremely rapid; an increase in IP_3 was detectable at 5 sec and peaked at 15-30 sec at approximately 300% of basal values. Stimulation than gradually declined returning almost to baseline by 2 min. IP_2 accumulation followed a similar pattern after a slight time delay. Although basal values of both IP_1 and IP_4 were higher than either IP_2 or IP_3, minimal stimulation in response to BK was seen over this time period. Low (1-10 mM) concentrations of LiCl appeared to have subtle effects on the kinetics of IP3 production/metabolism in response to BK, slightly delaying the time point of peak accumulation. Thus at earlier times, Li appeared to inhibit IP3 while after longer stimulation, the response was equal or slightly potentiated.

401.10

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

Bradykinin (Bk) increases inositol tris-phosphate ($InsP_3$) in cultured dorsal root ganglion (DRG) neurones. Serotonin also increased $InsP_3$ formation in these cultures. The responses to maximally effective concentrations of the two agonists were not additive, suggesting that they act on the same population of neurones. Pretreatment of the neurones with Bk, followed by its removal, resulted in a decreased response to a second application of the peptide. The extent of this decrease was dependent on the desensitising concentration of Bk (IC_{50} value 30nM) and the duration of the pretreatment. Pretreatment with 30nM Bk caused both a five-fold shift to the right of the concentration-response curve for Bk-induced $InsP_3$ formation (EC_{50} increased to value 30nM) and a 50% reduction in the maximum response. Pretreatment with Bk had no effect on subsequent responsiveness to serotonin, likewise pretreatment with serotonin did not affect the responsiveness to Bk. After Bk pretreatment, a prolonged incubation (60min) restored responsiveness of the cells to Bk. Exposure of the cultures to dibutyrl cGMP or 4β-phorbol 12,13-dibutyrate (PDBu) also inhibited Bk-induced $InsP_3$ formation, causing a rightward shift of the Bk concentration-response relationship and a decrease in the maximum response. These findings indicate that Bk induces homologous desensitisation of inositol poyphosphate formation in DRG neurones which may involve cGMP-dependent kinase or protein kinase C.

401.11

LONG-TERM ACTIVATION OF PROTEIN KINASE C BY ANGIOTENSIN IN BOVINE ADRENAL MEDULLARY CELLS. R.K. Tuominen*, J. S. Hong and M. K. Stachowiak, (SPON: L. Lazarus). LMIN/NIEHS, Research Triangle Park, NC 27709.

Angiotensin II (AII) increases tyrosine hydroxylase and proenkephalin A mRNA levels in cultured bovine adrenal medullary (BAM) cells. The increases are inhibited by sphingosine, suggesting the involvement of protein kinase C (PKC). However, a 30 min incubation with AII, which activates PKC, is not sufficient to change the mRNA levels. Therefore, we examined whether long-term incubation with AII affects PKC. AII (200 nM) increased the particulate PKC activity by 50% and 58% after 6 and 18 hrs, respectively. Similar changes were produced by 2 nM AII at 12 hrs. Total (soluble + particulate) PKC activity was increased by 15% and 30% at 12 and 18 hrs. To determine the time required to induce changes in mRNA levels, cells were incubated with AII (20 nM) for 24 hrs and at 3, 6, 12 or 18 hrs saralasin (2 uM) was added. Twelve, but not 3 or 6, hr stimulation of AII receptors was sufficient to increase the mRNA levels. Thus, in addition to a short-term activation, AII induces a long-term increase in PKC activity. The long-term activation may mediate the effects of AII on the gene expression.

401.12

MECHANISM BY WHICH SIGMA LIGANDS INHIBIT STIMULATION OF PHOSPHOINOSITIDE METABOLISM BY MUSCARINIC CHOLINERGIC AGONISTS. P. Tolentino* and W.D. Bowen (SPON: R. Patrick). Div. Biol. & Med., Brown Univ., Providence, RI 02912.

We have previously reported that sigma ligands attenuate the ability of the cholinergic agonist, carbachol, to stimulate phosphoinositide (PPI) turnover in rat brain synaptoneurosomes (Eur. J. Pharmacol., 149:399, 1988). Here we investigate the mechanism of this effect. Synaptoneurosomes were prelabeled with [^{3}H]inositol, and [^{3}H]inositol phosphates collected after exposure to test compounds. (+)-Pentazocine and ditolylguanidine reduced the maximal stimulation by carbachol without affecting the ED_{50}, suggesting a non-competitive mechanism. Ligand concentrations 15-fold higher were required to attenuate norepinephrine-stimulated PPI turnover. Although some sigma ligands inhibited [^{3}H]QNB binding to muscarinic receptors at high concentrations, there was no correlation with potency in the PPI assay. (+)-Pentazocine reduced formation of all three [^{3}H]inositol phosphates and had no effect on their hydrolysis. When present during prelabeling, (+)-pentazocine did not affect incorporation of [^{3}H]inositol into the total inositol lipid pool. The differential sensitivity of muscarinic and adrenergic stimulation, and lack of effect at other points in the PPI pathway, suggests desensitization of muscarinic receptors.

401.13

LOSS OF MUSCARINIC RECEPTORS AND OF GUANINE-NUCLEOTIDE-STIMULATED PPI HYDROLYSIS IN HUMAN NEUROBLASTOMA CELLS UPON LONG-TERM TREATMENT WITH TPA OR MEZEREIN. C.L. Cioffi and S.K. Fisher. Neuroscience Laboratory, University of Michigan, Ann Arbor, MI 48104.

The actions of tumor promoters on the coupling of muscarinic receptors to hydrolysis of inositol lipids was investigated in human neuroblastoma SH-SY5Y cells. Addition of 50 nM 12-O-tetradecanoylphorbol (TPA) to these cells for 5 days led to neuronal differentiation, a 28% loss of specific [^{3}H]N-methylscopolamine binding and a consistently larger reduction (48%) in agonist-stimulated [^{3}H]-inositol phosphate generation. While mezerein could mimic the effect produced by TPA, the biologically-inactive 4α-phorbol-12,13-didecanoate was without effect on cell morphology, antagonist binding or phosphoinositide (PPI) turnover. Pretreatment with TPA or mezerein also led to a decline in the agonist-mediated rise in cytoplasmic [Ca^{++}] and a loss of protein kinase C activity. The ability of fluoride to induce [^{3}H]inositol phosphate release was significantly reduced in SH-SY5Y cells treated with TPA or mezerein. Treatment with these agents also impaired [^{3}H]-inositol phosphate formation induced by GTPγS or carbachol in digitonin-permeabilized cells. These results suggest that TPA and mezerein decrease PPI hydrolysis by both a reduction in muscarinic receptor number and inhibition of guanine-nucleotide-stimulated PPI turnover. (Supported by NIMH Grant MH42652 and NIMH Training Grant MH15794).

401.14

THE REGULATION OF PHOSPHATIDYLINOSITOL TURNOVER BY CYCLIC AMP MAY INVOLVE PRODUCTS OF ARACHIDONIC ACID METABOLISM. J. B. Schachter[1] and B. B. Wolfe[2]. Depts. of Pharmacology, [1]Univ. of Pennsylvania, Philadelphia, PA. 19104. and [2]Georgetown Univ., Washington, D.C. 20007.

The activation of adenosine A_1 receptors in DDT_1 smooth muscle cells resulted in a dramatic reduction of cyclic AMP (cAMP) accumulation. In addition to this reduction of cAMP accumulation, activation of adenosine A_1 receptors resulted in a potentiation of phoshatidylinositol (PI) turnover. While the adenosine A_1 selective agonist, cyclopentyladenosine (CPA) had no effect upon PI turnover by itself, it caused nearly a twofold increase in norepinephrine-stimulated (NE-stimulated) PI turnover. To ascertain whether the effect of CPA on NE-stimulated PI turnover was related to the effect of CPA on cAMP accumulation, 8-bromo cAMP (8-BrcAMP) was added exogenously. While 8-BrcAMP had no effect upon NE-stimulated PI turnover, the CPA-induced potentiation of NE-stimulated PI turnover was completely antagonized. The potentiation of PI turnover by CPA was also blocked by pretreating cells with pertussis toxin, an agent which blocks the inhibition of cAMP accumulation by CPA.

In addition to being regulated by cAMP, the potentiation of PI turnover by CPA was calcium dependent and was antagonized by inhibitors of phospholipase A2 and by inhibitors of lipoxygenase. Thus, cAMP may regulate PI turnover by regulating the production of products of the lipoxygenase pathway which stimulate PI turnover. (HL-07502 and GM-31155)

401.15

MECHANISM OF K-INDUCED ACTIVATION OF PROTEIN KINASE C. A. Bhave*, S.V. Bhave*, R.K. Malhotra*, T.D. Wakade* and Arun R. Wakade.(SPON.: J. Benjamins), Dept. of Pharmacol., Wayne State Univ., Detroit, MI 48201.

Increasing concentrations of KCl (17.5 to 75 mM) caused concentration-dependent activation of protein kinase C (PKC) in brain, adrenal medulla and cardiac cell cultures and sympathetic neurons (SN). Extent of PKC activation by 35 mM K was comparable to that seen with 100 nM phorbol 12, 13-dibutyrate or 30 uM acetylcholine (ACh). Biochemical pathway responsible for activation of PKC was studied in homogeneous cultures of chick embryonic SN. 35 mM K did not stimulate formation of ^{3}H-inositol phosphates in ^{3}H-myo-inositol loaded-SN but produced a significant increase (160%) in 1,2-diacylglycerol (DAG) content. ACh enhanced ^{3}H-inositol monophosphate formation (1500%) and (DAG) content (165%). Turnover of ^{3}H-choline was facilitated by 35 mM K but not by ACh. Qualitatively similar results were obtained with 35 mM K and ACh on DAG and ^{3}H-inositol monophosphate formation in the rat adrenal medulla. These data suggest that depolarizing stimulus activates phosphatidylcholine pathway to generate DAG whereas ACh acts via phosphatidylinositol pathway to produce DAG for activation of PKC.

401.16

METABOLISM OF INOSITOL 1,3,4,5-TETRAKISPHOSPHATE IN RAT BRAIN HOMOGENATES. P. Kurian*, L. J. Chandler* and F.T. Crews. Department of Pharmacology, Univ. of Florida College of Medicine, Box J-267, Gainesville, FL 32610.

A number of hormones, growth factors and neurotransmitters have been shown to stimulate the hydrolysis of membrane phosphoinositides, resulting in the formation of Ins(1,4,5)P_3 and other inositol phosphates. It is now clear that inositol phosphate metabolism is a complicated process that may be highly regulated. In addition to being dephosphorylated by the action of a 5-phosphatase, Ins(1,4,5)P_3 can be phosphorylated by Ins(1,4,5)P_3 3-kinase to form to Ins(1,3,4,5)P_4. Although the physiological significance of the higher inositol polyphosphates is not clear, recent evidence suggest that Ins(1,3,4,5)P_4 may also have second messenger function. To more fully understand the disposition of Ins(1,3,4,5)P_4 in brain, we investigated the metabolism of Ins(1,3,4,5)P_4 by adding [^{3}H]Ins(1,3,4,5)P_4 to rat whole brain homogenates resuspended in physiological buffers. Analysis of the inositol phosphate metabolites of Ins(1,3,4,5)P_4 by h.p.l.c. showed that [^{3}H]Ins(1,3,4,5)P_4 was first dephosphorylated to Ins(1,3,4)P_3 and then to Ins(1,3)P_2 and Ins(3,4)P_2. Three inositol monophosphates {Ins(1)P, Ins(3)P and Ins(4)P} were formed. Since no Ins(1,4)P_2 was detected, we concluded that Ins(1)P was formed from Ins(1,3)P_2, while Ins(4)P was formed from Ins(3,4)P_2. Ins(3)P came from Ins(1,3)P_2 and/or Ins(3,4)P_2. We report here an ion-exchange h.p.l.c. gradient system that separates all of these isomers in a single run.

401.17

PHOSPHOLIPASE D AND PHOSPHOLIPASE C IN HUMAN CHOLINERGIC NEUROBLASTOMA (LA-N-2) CELLS -MODULATION BY MUSCARINIC AGONISTS AND PROTEIN KINASE C-

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Stimulation of muscarinic receptors has been demonstrated to activate a phospholipase C [PLC, acting on inositol phospholipids, e.g. Brown, Brown-Masters, 1984, Trends Pharmacol. Sci. 5:417-419] and a phospholipase D [PLD, acting on phosphatidylcholine, Lindmar et al., 1988, Biochem. Pharmacol. 37:4689-4695]. The present study was aimed to characterize the modulation of PLD and PLC by muscarinic receptor activation in a cholinergic cell line, the human neuroblastoma LA-N-2 cells (e.g. Richardson et al., 1989, Brain Res. 476:323-331). LA-N-2 cells were prelabelled with [^{3}H]-inositol and [^{3}H]-oleic acid and then stimulated with the muscarinic agonist carbachol (CCh). When incubated in the presence of ethanol and lithium, CCh caused a time- and dose-dependent, atropine and pirenzepine sensitive formation of [^{3}H]-inositol phosphates, [^{3}H]-phosphatidic acid (PtdA) and [^{3}H]-phosphatidylethanol (Peth). The EC_{50}-values for CCh were about 30µM. Since the biosynthesis of Peth is considered as a specific indicator for PLD-activity (e.g. Kobayashi, Kanfer, 1987, J. Neurochem. 48:1597-1603) these data suggest an activation of PLD in LA-N-2 cells by muscarinic agonists. Activation of protein kinase C (PKC) is known to stimulate PLD (e.g. Liscovitch, 1989, J. Biol. Chem. 264:1450-1456). In LA-N-2 cells the PKC-activator, 12-O-tetradecanoylphorbol-13-acetate (TPA), stimulated the formation of PtdA and Peth but attenuated the CCh-induced formation of inositol phosphates. In contrast, the effects of TPA and CCh on the production of Peth were additive. The present data provide evidence that muscarinic receptors in LA-N-2 cells are coupled to both PLC and PLD. The activation of both enzymes is inversely regulated by PKC, raising the possibility that PLD may participate in signal transduction mechanisms either independently of the phosphoinositide-turnover or by modulating the initial "PI"-signal.

(Supported by NIH grant MH-28783 and by a postdoctoral fellowship of the Deutsche Forschungsgemeinschaft to J.S.)

401.18

BRADYKININ AND CARBACHOL INCREASE PHOSPHOLIPASE D ACTIVITY IN PC12 CELLS. J. HORWITZ* (SPON: R.L. PERLMAN) Dept. of Pediatrics and Kennedy Mental Retardation Res. Ctr., The University of Chicago, Chicago, IL 60637

Bradykinin and carbachol increased the production of both [^{3}H]phosphatidic acid ([^{3}H]PA) and [^{3}H]diacylglycerol in PC12 pheochromocytoma cells prelabeled with [^{3}H]arachidonic acid. [^{3}H]PA was elevated within 30 sec, whereas [^{3}H]diacylglycerol did not increase until 2 min. This time course suggested that the increase in PA could be due to an activation of phospholipase D. This enzyme also catalyzes the transfer of phosphatidyl groups to various acceptors; in the presence of ethanol, this transphosphatidylation produces phosphatidylethanol. When cells were prelabeled with [^{3}H]palmitic acid and then incubated with 5% ethanol, bradykinin caused a significant increase in the accumulation of [^{3}H]phosphatidylethanol. The phospholipid source of the phosphatidic acid was also investigated. Different classes of phospholipids contain different proportions of stearic acid and palmitic acid. When cells were labeled with [^{14}C]stearic acid and [^{3}H]palmitic acid and then stimulated with bradykinin, the [^{14}C]/[^{3}H] ratio of the increment in PA was intermediate between the ratios of phosphatidylcholine and phosphatidylinositol; when cells were stimulated with carbachol, the ratio was similar to the [^{14}C]/[^{3}H] ratio in phosphatidylcholine. Bradykinin and carbachol may activate a phospholipase D that catalyzes the formation of PA from phosphatidylcholine and other phospholipids. (Supported by NS-22694 and HD-04583)

401.19

STIMULATION OF CHOLINE PHOSPHOLIPID HYDROLYSIS AND PHOSPHORYLCHOLINE PRODUCTION IS MEDIATED BY PROTEIN KINASES A AND C IN RAT ANTERIOR PITUITARY CELLS *IN VITRO*.

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Recent evidence obtained in a variety of transformed cell lines has suggested a role for the hydrolysis of the choline-based phospholipids phosphatidylcholine (PtdCho) and sphingomyelin (SphMyl) in signal transduction; this study addressed the presence of such a system in primary cultures of female rat anterior pituitary cells.

[^{3}H]Choline-prelabeled cell cultures were exposed either to phorbol myristate acetate (PMA; 0.1-100 nM) or to dibutyryladenosine cyclic monophosphate (dbcAMP; 0.01-10 mM) for varying times. Both of these treatments produced time- and concentration-related increases in the extracellular accumulation of [^{3}H]phosphorylcholine ([^{3}H]PCho); respective EC_{50} values for PMA and dbcAMP were 0.1 nM and 1.0 mM during a 45-min exposure period. No significant changes in intracellular levels of [^{3}H]PCho were observed. Parallel reductions in cellular [^{3}H]PtdCho and [^{3}H]SphMyl levels accompanied these responses in both instances.

Choline phospholipid breakdown was also stimulated by neuropeptides known to promote prolactin release. Increased accumulation of [^{3}H]PCho in the medium was elicited by 45-min exposures to 100 nM thyrotropin-releasing hormone (229%), angiotensin II (210%), neurotensin (171%), bombesin (276%), or vasoactive intestinal peptide (367%). As before, intracellular [^{3}H]PCho levels were not significantly modified.

These data indicate that choline phospholipid hydrolysis accompanies receptor activation in the anterior pituitary, and may be mediated by the independent actions of protein kinases A and C. We suggest that this process may participate in the regulation of prolactin release.

[This work was supported in part by NIH grant CA 07535-25 (R.M.M.).]

401.20

SPHINGOSINE ACTIVATES PHOSPHOLIPASE D IN NG108-15 CELLS. M. Liscovitch* and Y. Lavie* (SPON: Eli Hazum). Dept. of Hormone Res., The Weizmann Inst. of Sci., Rehovot, Israel.

Sphingosine and other lysosphingolipids accumulate in tissues of patients with inherited lysosomal storage diseases collectively called sphingolipidoses. Although it has been suggested that lysosphingolipid accumulation plays a major role in the pathophysiology of these diseases, the mechanism(s) of their cytotoxic action remained unidentified. We here demonstrate that sphingosine, as well as some other long chain bases, are potent activators of phospholipase D (PL-D) in neural-derived NG108-15 cells. Sphingosine caused severalfold stimulation of phosphatidic acid and phosphatidylethanol formation. (The latter is an unusual phospholipid which is characteristically produced by PL-D when ethanol acts as the phosphatidyl group acceptor.) Sphingosine activation was concentration-dependent with a half-maximal effect observed at 15 µM and 40 µM, in the absence and presence of equimolar BSA, respectively. Activation of PL-D preceded the development of the acute cytolytic response to sphingosine. Galactosylsphingosine (psychosine) and octadecylamine (stearylamine) mimicked the action of sphingosine on PL-D activity. Octylamine and 1-octadecanol (stearylalcohol) were inactive, suggesting that a long aliphatic chain and the terminal amino group are important structural requirements for the activation of the enzyme. PL-D may thus constitute an important cellular target for sphingosine action.

401.21

EFFECTS OF INTRATHECALLY ADMINISTERED ISOQUINOLINESULFONAMIDES H7, H8 AND HA1004 ON ACOUSTIC STARTLE; N. Boulis, and M. Davis, Dept of Psychiatry, Yale Univ. Sch. of Med., 34 Park St., New Haven, CT, 06508.

The spinal synapse of the acoustic startle reflex is subject to several forms of plasticity. The present study sought to evaluate the role of two second messengers at this site. The isoquinolinesulfonamide H7, and the ganglioside GT1b, which inhibit protein kinase C activation, were infused intrathecally into the subarachnoid space of the lumbar spinal cord. H7 caused a marked, dose-dependent (10-300 μg) amplification of the startle reflex in comparison with its vehicle ($p<.001$). Like H7, GT1b (40 μg) produced a marked increase in the startle reflex versus its vehicle ($p<.03$). HA1004 (150 μg) and H8 (130 μg), which have substantially less C kinase inhibitory activity than H7, caused only modest increases in startle.

The isoquinolinesulfonamides have also been shown to inhibit A kinase activity. Of the group, H8 is the most effective. Previous work has shown that drugs thought to increase cAMP increase the startle response when infused intrathecally. In a second study, fifteen minutes after either H8 (130 μg) or vehicle infusions, animals received 50 μg of dibutyryl cAMP. By itself H8 had little effect on startle but completely blocked the normal excitatory effect of dibutyryl cAMP on startle ($p<.001$). The pharmacological specificity of H8 was shown by the fact that dibutyryl cAMP continues to elevate startle ($p<.006$) in the presence of H7. Further, the inability of H8 to inhibit the excitatory effect of intrathecal 8-OH-DPAT infusions ($p<.19$) demonstrates H8's behavioral specificity. Thus, while H8 inhibits A kinase-dependent increases in startle, it does not inhibit all increases in startle. Further, as evidenced by the primary increases in startle caused by intrathecal infusions of the isoquinolinesulfonamides and the ganglioside GT1b, spinal PKC inhibition elevates startle, perhaps because PKC tonically inhibits startle at the spinal level.

CATECHOLAMINES: NEUROTOXICITY

402.1

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

Systemic injections of the neurotoxin DSP-4 produce depletion of norepinephrine (NE) and dopamine-ß-hydroxylase (DBH) in brain regions innervated by the LC. We have studied the effects of DSP-4 on the morphology of central NE axons over a 2 week period using antibodies to NE and to DBH. Rats were sacrificed at daily intervals following a single dose of DSP-4 (i.p., 50 mg/kg; gift of Dr. S.B. Ross). One day after DSP-4, staining with anti-NE revealed extensive loss of transmitter in LC axons. By contrast, there was no change in NE axon staining with anti-DBH until day 4. Thereafter, an abrupt loss of DBH staining was observed in brain regions innervated by the LC. This loss of LC axon staining coincided with the appearance of morphologically altered axons. These fibers were characterized by their thick diameter and intense staining; some of these axons showed prominent swellings and had numerous short branches, resembling axonal sprouts. They were most frequent in the deep layers of cortex and along ascending pathways of NE axons. These remaining fibers could be visualized with both antisera between days 4 and 10. By day 14, there was no staining with either anti-NE or anti-DBH in brain regions innervated by the LC.

We propose that the sequence of events following DSP-4 treatment can be divided into two phases: initially, there is rapid and profound loss of transmitter from LC axons; this phase is followed by morphological alterations of fibers and disappearance of staining. We suggest that the transient appearance of swollen axons and axonal sprouts and the loss of DBH staining mark the onset of LC axon degeneration. [Support NIH grant MH 41977]

402.2

DSP-4 HAS DIFFERENT AFFINITIES FOR THE NOREPINEPHRINE (NE) CARRIER IN CEREBRAL CORTEX AND HYPOTHALAMUS. R. Grzanna, R. Zaczek, J.-M. Fritschy, S. Culp* and E.B. De Souza. Department of Neuroscience Johns Hopkins University School of Medicine and NIDA, ARC, Baltimore, Md. 21205

A single systemic injection of the NE neurotoxin DSP-4 causes profound reductions in NE levels and NE-uptake in cerebral cortex but not in the hypothalamus. DSP-4 is thought exert its effects by binding selectively and irreversibly to the NE uptake carrier. In this study we tested the hypothesis that the differential effects of DSP-4 on NE axons in cerebral cortex and hypothalamus are due to differences in the affinity of DSP-4 for the NE uptake carrier. We have measured NE uptake into synaptosomes isolated from cerebral cortex and hypothalamus in the presence of 100 and 300 nM DSP-4 (gift of Dr. S.B. Ross). Uptake assays were conducted over a 5 min. period with 1 μM desipramine to assess non-specific incorporation of NE. At 100 nM DSP-4 inhibited NE uptake into cortical synaptosomes by 30% but had no effect on NE uptake into hypothalamic synaptosomes. At 300 nM DSP-4 produced a substantial increases in the Km of NE uptake into synaptosomes from both brain regions but caused only minor alterations in the Vmax indicating that the drug is a competitive inhibitor of NE uptake. DSP-4 had a 2.6-fold higher affinity ($p < 0.01$) for the NE uptake into cortical synaptosomes (K_i = 180 ± 38 nM) compared to hypothalamic synaptosomes (K_i = 460 ± 36 nM). The results suggest that the differential neurotoxic effects of DSP-4 in various brain regions may be related to differences in the affinities of the drug for the NE uptake carrier. [Support NIH grant MH 41977]

402.3

CORTICAL AND HYPOTHALAMIC NOREPINEPHRINE (NE) TRANSPORT PROCESSES DIFFER IN THEIR KINETIC AND PHARMACOLOGIC PROPERTIES. S. Culp*, R. Grzanna, E.B. De Souza, J.-M. Fritschy and R. Zaczek (SPON. M. Steinmetz). NIDA/ARC and Dept. Neurosci., Johns Hopkins Univ. School of Medicine, Baltimore, MD 21224.

Differential effects of the neurotoxin DSP-4 in rat hypothalamus and cortex led us to propose that NE uptake processes in these two areas may differ. We examined sodium dependent uptake of ^{3}H-NE into rat brain synaptosomes from cortex and hypothalamus using 1.0 μM desipramine (DMI) to measure non-specific ^{3}H-NE incorporation. Saturation studies revealed that NE possessed a significantly ($p < 0.05$) higher affinity for uptake in cortex (K_m = 39.5 ± 7.5 nM) than in hypothalamus (K_m = 100.3 ± 12.1 nM). The maximal velocity of ^{3}H-NE uptake was two fold higher in hypothalamus (V_{max} = 2.1 ± 0.1 nmoles/mg protein/min) than that observed in cortex (V_{max} = 1.1 ± 0.1 nmoles/mg protein/min). Preliminary pharmacological studies indicated that nisoxetine was equally potent at inhibiting cortical and hypothalamic ^{3}H-NE uptake processes (K_i cortex = 1.48 ± 0.09 nM; K_i hypothalamus = 1.34 ± 0.16 nM). This was also true for DMI (K_i cortex = 1.23 ± 0.09 nM; K_i hypothalamus = 1.17 ± 0.16 nM). However, DSP-4 was a more potent inhibitor of ^{3}H-NE uptake in cortex than in hypothalamus, with inhibition constants of 180 ± 38 nM and 460 ± 36 nM in these two areas, respectively ($p < 0.01$). On the other hand, mazindol possessed a higher affinity in hypothalamus (K_i = 0.30 ± 0.08 nM) than in cortex (K_i = 0.55 ± 0.05 nM; $p < 0.05$). These results suggest that NE is taken up by different carrier proteins in cortex and hypothalamus. Pharmacological differences in these carriers may be exploited to develop more specific therapeutic agents. [Support NIH grant MH 41977]

402.4

CHARACTERIZATION OF THE ORIGINS OF ASTROCYTE RESPONSE TO INJURY USING THE DOPAMINERGIC NEUROTOXICANT, MPTP. J.P. O'Callaghan, D.B. Miller and J.F. Reinhard[1]. U.S. Environmental Protection Agency and [1]The Wellcome Research Laboratories, Research Triangle Park, NC 27711.

Astrocytes hypertrophy following a variety of nervous system insults and the hallmark of this response is an enhanced expression of the astrocyte intermediate filament protein, glial fibrillary acidic protein (GFAP). 1-Methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) causes degeneration of the nigrostriatal pathway, an effect which requires the MAO-B catalyzed formation of the 1-methyl-4-phenylpyridinium species (MPP^+) and its dopamine-carrier mediated uptake into nigrostriatal neurons. To characterize the origins of astrocyte reaction to injury, we administered MPTP (0-25 mg/kg, sc) to C57BL/6J mice and then assayed striatal GFAP. We found that MPTP caused a rapid and large increase in GFAP. This effect was completely blocked by inhibitors of MAO B and the dopamine uptake system but not by blockers of MAO A. Blood-borne or brain-derived interleukin-I did not appear to mediate the effect of MPTP on GFAP. The data indicate that factors derived from the dopamine terminal initiate the astrocyte response to MPTP.

402.5

EFFECTS OF THE MONOAMINERGIC NEUROTOXICANT, MPTP, IN C57BL/6J FEMALE MICE OF DIFFERENT AGES. D.B. Miller[1], J.P. O'Callaghan[1], L. Manzino*, B.A. Sieber*, A.Giovanni* & R.E. Heikkila*. [1]U.S. Environmental Protection Agency, Research Triangle Pk., NC, 27711 and *UMDNJ-Robert Wood Johnson Medical School, Piscataway, NJ 08854.

1-Methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) causes degeneration of the dopaminergic nigrostriatal pathway in experimental animals including C57BL/6 mice. MPTP-induced neurotoxicity is dependent upon the monoamine oxidase-B (MAO-B) catalyzed formation of the 1-methyl-4-phenylpyridinium species (MPP^+) from MPTP. Here, we measured neostriatal levels of MAO-B in naive female C57BL/6J mice (3,6.5, 11 and 18 months of age). Age-related effects of a single dose of MPTP (15 mg/kg, s.c.) were determined by measuring neostriatal levels of MPP^+, dopamine and glial fibrillary acidic protein (GFAP). GFAP, the major intermediate filament protein of astrocytes, increases after CNS damage induced by MPTP and other neurotoxicants. The youngest group had lower levels of MAO-B and GFAP. After MPTP the 3-month old mice had 1) lower levels of neostriatal MPP^+, 2) less depletion of neostriatal dopamine and 3) lower levels of neostriatal GFAP relative to the other groups. Data are consistent with previous reports of age-related MPTP neurotoxicity.

402.6

STUDIES ON THE MECHANISM OF MPP^+-INDUCED NEUROTOXICITY. S.K. Youngster*[i], R.E. Heikkila[1], N. Castagnoli, Jr.*[2], and H. Rollema[3] (SPON: P. Manowitz). [1]Neurology Dept., UMDNJ-Robert Wood Johnson Medical School, Piscataway, NJ 08854, [2]Chemistry Dept., Virginia Polytechnic and State University, Blacksburg, VA 24061, [3]Dept. of Medicinal Chemistry, State University Groningen, 9713 AW Groningen, The Netherlands.

The 1-methyl-4-phenylpyridinium ion (MPP^+) resulting from the monoamine oxidase (MAO)-catalyzed oxidation of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) is the actual neurotoxic species following systemic administration of MPTP to experimental animals. We have used in vivo dialysis of dopamine (DA) to determine the relative potencies of MPP^+ and several of its analogs as neostriatal dopaminergic neurotoxins in rats. These analogs were the predicted 4-electron oxidation products which would be formed via the actions of MAO on structural analogs of MPTP. The capacities of the compounds to be taken up by the neostriatal DA transport system and to inhibit respiration in isolated mitochondrial preparations were also determined. The correlation that exists between the abilities of the analogs to destroy DA neurons and their capacities to act as substrates for the DA carrier and inhibitors of mitochondrial respiration suggests that these parameters are important determinants in the toxicity of MPP^+ and some of its analogs.

402.7

EFFECT OF ALTERATION OF ENERGY METABOLISM ON 2'ET-MPTP-INDUCED TOXICITY IN PC12 CELLS. A.N. Basma*, R.E. Heikkila, H.M. Geller and W.J. Nicklas. (SPON: R.C. Duvoisin) Depts. of Neurology and Pharmacology UMDNJ-Robert Wood Johnson Medical School, Piscataway, NJ, 08854.

2'Et-MPTP is, like MPTP, a potent dopaminergic neurotoxin. It has been proposed that 2'Et-MPTP-promoted neuronal death is a consequence of 2'Et-MPP^+-induced inhibition of mitochondrial respiration. In the present study, we have examined the mechanism of 2'Et-MPTP-induced toxicity in PC12 cells, which are neoplastic in nature and have a high rate of anaerobic glycolysis with a large production of lactate and a low utilization of glucose carbon through the Krebs cycle. We compared the effects of varying concentrations of 2'Et-MPTP on PC12 cells grown in RPMI 1640 medium supplemented with either glucose or with pyruvate. The latter substrate is directly metabolized via the Krebs cycle. 2'Et-MPTP was more toxic to PC12 cells grown in the pyruvate- supplemented RPMI 1640 medium than to cells grown in glucose-supplemented medium. For example after two days, 50 μM 2'Et-MPTP killed 69% of cells in the pyruvate medium but only 11% of cells in the glucose medium. These results demonstrate that an alteration in the energy metabolism of the cells can affect their susceptibility to 2'Et-MPTP. Results of these studies and reasons for this differential toxicity will be discussed.

402.8

MPTP-INDUCED NEUROTOXICITY IN THE RAT. A. Giovanni, P.K. Sonsalla, and R.E. Heikkila. Dept. of Neurology, UMDNJ-Robert Wood Johnson Medical School, Piscataway, NJ 08854.

The rat demonstrates relative insensitivity to the neurotoxic actions of MPTP. The LD_{50} of MPTP in the rat is lower than the dose needed to produce marked dopaminergic neurotoxicity. This is perhaps the major difficulty in the ability to induce a parkinsonian syndrome in the rat. We have found that pretreatment with guanethidine, a peripheral adrenergic depleting agent, substantially decreases mortality in the rat in response to large doses of MPTP (60 mg/kg s.c.) without directly affecting the neurotoxic actions of MPTP. In the present study, MPTP was given in combination with guanethidine pretreatment to successfully induce dopaminergic neurotoxicity in the rat. This treatment resulted in a marked depletion of dopamine (DA) and its metabolites and loss of tyrosine hydroxylase (TH) activity in the rat caudate nucleus. Additionally, MPTP induced a significant but slightly less pronounced decrement of DA and TH activity in the substantia nigra of the rat. The relationship between the loss of DA and TH activity in the caudate to the loss of 1) DA, 2) TH activity and 3) dopaminergic neurons in the substantia nigra will be discussed.

402.9

DOPAMINERGIC NEUROTOXICITY OF MPTP ANALOGS. R.E. Heikkila, P.K. Sonsalla, I. Terleckyj* and S.K. Youngster*. Dept. of Neurology, UMDNJ-Robert Wood Johnson Medical School, Piscataway, NJ 08854.

1-Methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) causes a degeneration of nigrostriatal dopaminergic neurons in monkeys and mice. Monoamine oxidase-B (MAO-B) catalyzes the oxidation of MPTP to 1-methyl-4-phenylpyridinium ion both in vitro and in vivo, and this oxidation is a necessary event in the neurotoxic process. In vitro, MAO-A oxidizes MPTP analogs having either a methyl or ethyl group in the 2'position and also plays a role in the bioactivation of these compounds to neurotoxins in vivo. In these studies we evaluated several analogs of MPTP for their abilities to be oxidized by MAO-A and MAO-B and to cause nigrostriatal dopaminergic neurotoxicity in mice. Several were found to be neurotoxic as evidenced by their capacities to cause reductions in the content of dopamine (DA) and its metabolites, binding of the DA uptake inhibitor 3H-GBR 12935, and tyrosine hydroxylase activity. Most of the neurotoxins were found to be bioactivated by MAO-A as well as MAO-B. Many of these compounds may turn out to be useful research tools.

402.10

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

Lesions of the dopaminergic innervation of the prefrontal cortex (PFC) affect indices of subcortical DA function (Pycock et al, Nature, 286:74-77, 1980). We investigated whether PFC lesions at 21 days of age would lead to greater perturbation of subcortical DA function than equivalent lesions sustained during adulthood. Twelve adult male (nine weeks old) and twelve male weanling (21 days old ±1) rats received 25 mg/Kg DMI (IP) prior to the bilateral infusion of 6-OHDA (4μg in 1μl in weanlings and 8μg in 2μl in adults) or the vehicle into the medial PFC. Levels of norepinephrine, dopamine and homovanillic acid were measured in the PFC, caudate nucleus (CPU), and n. accumbens (ACB) 7 days later. DA and NE in the PFC were significantly ($ps<0.01$) reduced in both age groups. Subcortically, levels of dopamine in the ACB were increased by 220% in weanling rats relative to the 142% increase seen in adult rats. DA activity in the CPU was unchanged. These data suggest that ontogenetic lesions of the PFC may lead to greater perturbation of subcortical DA function than similar lesions sustained in adulthood.

402.11

DOPAMINE D1 AND D2 RECEPTORS IN THE RAT CAUDATE-PUTAMEN (CPu): FUNCTIONAL SENSITIZATION AND LOSS OF D1-MEDIATED ENABLING BY 6-OHDA PRETREATMENT X.-T. Hu, M.P. Galloway, and F.J. White, Dept. Psychiatry, Neuropsychopharmacol. Lab., Wayne St. Univ. Sch. Med., Lafayette Clinic, Detroit, MI 48207

Extracellular single unit recording and microiontophoretic techniques were used to determine the sensitivity and interaction of D1/D2 receptors in the CPu of rats which were pretreated with either 6-OHDA (100 g/5 l, LV), ä-methyl-p-tyrosine (AMPT, 500mg/kg, ip) or 6-OHDA plus AMPT. Seven-ten days after 6-OHDA, CPu DA levels were reduced by 88%. Cells/tracks analysis revealed more spontaneously active CPu cells in 6-OHDA-lesioned rats. Current-response curves exhibited significantly enhanced inhibitory responses of CPu cells to both the selective D1 agonist SKF-38393 (SKF) and D2 agonist quinpirole (Quin). However, the potentiation of Quin-induced inhibition by co-ejected SKF observed in controls was abolished by 6-OHDA pretreatment. Moreover, the absolute requirement for SKF to enable Quin's effects in unlesioned rats acutely depleted (80%) of DA with AMPT was also eliminated by 6-OHDA (with and without AMPT). These findings indicate that 6-OHDA lesions result in (1) the functional sensitization of both D1 and D2 receptors and (2) the loss of D1-mediated enabling of D2 responses in the rat CPu (Supported by APDA, USPHS Grants DA-04093, and MH-40832 to FJW).

402.12

NGF POTENTIATES THE EFFECTS OF 6-OHDA-INDUCED NIGRAL LESION ON STRIATAL PROENKEPHALIN AND PROTACHYKININ mRNA IN RAT BRAIN. J.L. Cadet, J. Angulo, V. Jackson-Lewis, B.S. McEwen, and S. Fahn. Columbia Univ., Dept. Neurology, NY, NY 10032 and Rockefeller Univ., NY, NY 10021.

Lesions of nigral dopaminergic cells with 6-hydroxydopamine (6-OHDA) cause increases in proenkephalin (PEK) mRNA but decreases in protachykinin (PTK) mRNA in rat striatum (Angulo et al. 1987). In the present study, we have investigated the possibility that nerve growth factor (NGF) may influence these changes. Adult rats received unilateral 6-OHDA-induced nigral lesions. The animals were then tested until they developed stable apomorphine (APO)-induced rotation. They were then divided into two groups according to rotational behavior and were given intraventricular infusions of either NGF or saline for 28 days. The rats were then sacrificed and the brain processed for in situ hybridization histochemistry. As previously reported, 6-OHDA nigral lesions caused increases in PEK mRNA but decreases in PTK mRNA in rat striatum. These changes were potentiated by chronic infusion of NGF. The potentiation was more evident on the dorsolateral aspect of the caudate putamen. However, in situ hybridization for tyrosine hydroxylase (TH) mRNA in the SN showed no differences between the groups. The data will be discussed in relation to the topography of striatal dopamine afferents from the SN.

402.13

IMMUNOHISTOLOGICAL CHARACTERIZATION OF MPTP INDUCED DEGENERATION IN STRIATUM AND SUBSTANTIA NIGRA OF C57 MICE. K. F. Jensen, D.B. Miller, J.P. O'Callaghan and J.F. Reinhard[1] Neurotoxicology Division, U.S. Environmental Protection Agency and [1]Wellcome Research Laboratories, Research Triangle Park, NC 27711.

MPTP induced damage to substantia nigra neurons has been hypothesized to occur as a result of axonopathy followed by retrograde degeneration. To address this issue we have used glial fibrillary acidic protein (GFAP) and tyrosine hydroxylase (TH) immunohistochemistry as well as a silver degeneration stain to characterize alterations occurring at the terminal fields within the striatum and neurons within substantia nigra. C57 mice where given 12.5, 25 or 40 mg/kg MPTP and sacrificed at intervals from 12 hours to 5 days postdosing. Marked increase in GFAP immunoreactivity occurs in striatum of MPTP animals at times when only slight increases were observed in substantia nigra. Increases in GFAP parallel decreases in TH immunoreactivity and evidence of silver degeneration. These results further characterize the vulnerability of striatal axons to MPTP.

CATECHOLAMINES III

403.1

COMPARISON OF THE PHARMACOLOGICAL RESPONSIVENESS OF DOPAMINE IN THE RAT MEDIAL PREFRONTAL CORTEX AND STRIATUM, B. Moghaddam and B.S. Bunney, Dept. of Pharmacology & Psychiatry, Yale University School of Medicine, New Haven, CT. 06510.

Using the technique of in vivo microdialysis combined with a small-bore liquid chromatography system, we have measured the basal and drug induced fluxes of extracellular dopamine (DA) in the medial prefrontal cortex (mPFC) of chloral hydrate anesthetized rats and have compared our findings in the mPFC to those observed in the striatum. The results were as follows: (1) At a flow rate of 2 µl/min, basal level of DA in the mPFC, was 0.32±0.1 (n=28) fmoles/µl perfusate, which was nearly an order of magnitude less than that obtained from striatum, (2) α-methyl-p-tyrosine (150 mg/kg i.v.) significantly decreased DA release in mPFC and striatum. The magnitude and duration of response were similar in both regions. (3) Local perfusion with 30 mM K^+ had a more profound effect on DA release in striatum than in mPFC, the K^+-induced release in both regions was significantly attenuated in the absence of Ca^{++}, (4) The anxiogenic beta carboline FG 7142 (15 mg/kg i.p.) enhanced the release of cortical DA by about 50% while it was without an effect in striatum. (5) Similar to striatum, amphetamine (1 mg/kg, i.v.) significantly elevated while reserpine (5 mg/kg i.p.) rapidly attenuated the dopamine level in mPFC. These studies provide direct evidence that DA release in mPFC is K^+ and Ca^{++} dependent and is sensitive to pharmacological manipulations. This work was supported by USPHS, Awards MH28849, MH25642 and MH14276.

403.2

INTRACELLULAR STUDY OF THE EFFECT OF NOREPINEPHRINE ON SOMATOSENSORY CORTICAL NEURONAL RESPONSE TO GABA. W. Liu, F.M. Sessler, C.S. Lin and B.D. Waterhouse, Dept. of Physiol. and Biophys., Hahnemann Univ., Phila., PA. 19102-1192.

Considerable evidence from extracellular recording studies suggests that norepinephrine (NE) may augment inhibitory synaptic transmission within local circuits of mammalian neocortex. For example, experiments in intact animals have shown that microiontophoresis of NE or activation of the coeruleo- cortical afferent pathway can enhance stimulus evoked or GABA-induced depressant responses of cortical neurons. Further studies using neocortical tissue slices have demonstrated that these noradrenergic modulatory actions involve the beta- adrenoceptor linked cyclic-AMP second messenger system. In the present investigation we have begun to examine the transmembrane electrophysiological events which are associated with NE and GABA interactions on somatosensory cortical cells. Intracellular recordings were made from somatosensory cortical neurons in a submerged tissue slice preparation. The resting membrane potential of the cortical neurons (N=24) examined was 68.7 $\pm$ 8.6 mV, with an input resistance of 33.1 $\pm$ 12 meghoms. Superfusion of GABA in concentrations greater than 0.5 mM produced a small decrease in membrane potential (5.6%) associated with a reduction in membrane resistance (24.2%). In four of five cells tested, superfusion of NE at concentrations producing little or no change in membrane potential was associated with an increase in input resistance. In 40% of the cases tested, superfusion of NE during GABA application produced a net increase in membrane conductance, suggesting noradrenergic facilitation of GABA mediated membrane action. (Supported by AFOSR-87-0138, NINCDS 18081 to B.D.W.)

403.3

NORADRENERGIC MODULATION OF CORTICAL NEURONAL EXCITABILITY: PHARMACOLOGIC CHARACTERIZATION IN TERMS OF ADRENERGIC AND AMINO ACID RECEPTOR SUBTYPES. R.D. Mouradian, F.M. Sessler and B.D. Waterhouse. Department of Physiology & Biophysics, Hahnemann University, Philadelphia, PA. 19102-1192

Previous studies from our laboratory indicate that under both in vivo and in vitro conditions norepinephrine (NE) can facilitate excitatory somatosensory cortical neuronal responses to threshold stimulation of afferent synaptic pathways or threshold iontophoretic doses of putative transmitters. Further characterization of these effects indicates that they are mediated by an alpha-1 type adrenoceptor which may be linked to intracellular activation of protein kinase C. Additional studies provided evidence of a noradrenergic "gating" action, whereby otherwise subthreshold iontophoretic doses of Glu evoked robust excitatory discharges but only during concomitant administration of NE. In each of these studies investigations, changes in the responsiveness of rat somatosensory cortical neurons to iontophoretic pulses of Glu were assessed by quantitative analysis of drug response histograms collected before, during and after NE, alpha agonist or phorbol ester microiontophoresis. Recent experiments in rat neocortical tissue slices have focused on which of the Glu receptor subtypes may be involved in these noradrenergic modulatory effects. Interactions between NE and iontophoretic doses of NMDA which were subthreshold for producing excitation resulted in "gated" responses similar to those observed with subthreshold Glu. NE was also capable of potentiating responses to threshold doses of NMDA. Overall, these data suggest that noradrenergic modulation of cortical neuronal excitability may be expressed as either an enhancement of threshold level spiking or as a shift in the ability of the membrane to respond to subliminal synaptic stimuli. A further goal is to determine the extent to which the other identified glutamate receptor subtypes are involved in either or both of these modulatory actions. (Supported by AFOSR-87-0138, NINCDS 18081 to B.D.W.)

403.4

MICROINJECTION OF LIDOCAINE, GABA, OR SYNAPTIC DECOUPLERS INTO THE VENTROLATERAL MEDULLA BLOCKS SCIATIC-EVOKED ACTIVATION OF LOCUS COERULEUS. C. Chiang and G. Aston-Jones, Div Behav Neurobiol, Dept Mental Hlth Sci, Hahnemann Univ, Philadelphia, PA 19102.

While it is well-documented that locus coeruleus (LC) is potently activated by footpinch or sciatic nerve stimulation, little is known about the circuit producing this sensory response. Recent work in our laboratory has identified the medullary nucleus paragigantocellularis (PGi) as the major excitatory afferent to LC. Here, we use reversible pharmacologic blockade to implicate PGi as a critical link in the pathway mediating sciatic activation of LC in rats.

Bipolar electrical stimulation of the contralateral sciatic nerve was presented through 26-gauge needles inserted in the hindpaw. Extracellular recordings from single LC neurons were obtained with micropipettes. All drugs were pressure ejected (125-400 nl over 2 min) through micropipettes (50-75 um tip).

Lidocaine HCl (2% in saline) attenuated sciatic-evoked activation of LC in 75/109 cells, with 35 complete but reversible blocks (10 min recovery). However, lidocaine disrupts impulses in fibers as well as somata. To verify that synapses within PGi are involved in this response, the inhibitory transmitter GABA, or a synaptic decoupling manganese/cadmium (Mn/Cd) cocktail, was microinjected into PGi. GABA (2 M) reduced the sciatic-evoked response of LC in 27/29 cells (7 complete blocks) and may act at GABA-A receptors as baclofen had no effect (0/25 cells). Mn/Cd (40 mM/20 mM or 20 mM/10 mM) also attenuated LC activation in 10/12 cells with 2 blocks. With each drug, the most effective injection placement for total blocks was ventromedial PGi; injections near this region produced attenuations. Vehicle injections (n=11) or control injection placements (inf olive, rostrolateral PGi, nuc tractus sol, facial nuc) were ineffective.

These results are consistent with previous findings that pharmacologic blockade of PGi-evoked LC activity also blocks LC sciatic responses (Ennis and Aston-Jones, J. Neurosci. 8: 3644), and support the view that sciatic and other sensory activations of LC are mediated through its major excitatory afferent, PGi. Supported by PHS grant NS24698 and ONR/AFOSR Contract N00014-86-K-0493.

403.5

ELECTROPHYSIOLOGICAL EVIDENCE FOR MEDULLARY ADRENERGIC INHIBITION OF RAT LOCUS COERULEUS. B. Astier*[1] and G. Aston-Jones (SPON: G. Sterling) Div Behav Neurobiol, Dept Mental Hlth Sci, Hahnemann Univ, Philadelphia, PA 19102.
[1]Lab Neuropharm, CNRS UMR 12, Fac Pharm, Univ C Bernard, Lyon, France.

Stimulation of the nucleus paragigantocellularis (PGi) in the rostral ventrolateral medulla induces a dual response in locus coeruleus (LC): most cells are excited and a few are inhibited (Ennis & Aston-Jones 1988). However, when PGi-evoked excitation of LC is blocked by excitatory amino acid (EAA) antagonists, nearly all LC neurons exhibited inhibitory responses (ibid).

Anatomic studies have demonstrated adrenergic (Ad) inputs to LC from PGi (Pieribone et al. 1988), and many studies have shown that adrenaline inhibits LC discharge via alpha$_2$ receptors. Thus, it is possible that PGi-induced inhibition of LC is mediated by this Ad pathway. To test this hypothesis we studied the effect of the alpha$_2$ antagonist idazoxan (Ida) on (i) inhibition of LC by PGi without pharmacologic pre-treatment, (ii) inhibition of LC by PGi after EAA antagonism, and (iii) inhibition of LC by stimulation of the Ad medullary bundle (MB).

Ida (0.5-1 mg/kg, iv) potently attenuated purely inhibitory responses obtained in 2/3 LC neurons after PGi stimulation; in the third cell this drug revealed an underlying excitatory response. Ida either iv (0.5-1 mg/kg) or microinfused into LC (2.5 ng in 50 nl) blocked (3/9 cells) or decreased (5/9 cells) inhibition following PGi stimulation revealed by EAA antagonism with kynurenic acid (0.53 umol, icv), in the last cell such inhibition was unaffected. Stimulation of the Ad MB area inhibited 14/22 LC cells by at least 50%; 4 cells were less potently inhibited and 4 cells were unaffected, but no cell was excited by MB stimulation. In one cell tested to date, Ida (0.5mg/kg, iv) attenuated such inhibition.

These results favor the hypothesis that inhibition of LC from PGi is mediated by a direct input from the Ad MB, and indicate that this MB pathway may selectively convey inhibitory control over LC. This conclusion is consistent with our additional finding (Van Bockstaele et al., this meeting) of multiple distinct pathways from PGi to LC. Supported by PHS grant NS24698 and ONR/AFOSR contract N00014-86-K-0493.

403.6

RESPONSES OF MESOLIMBIC UNITS TO SENSORY INPUT IN RATS CONDITIONED BY REINFORCING BRAIN STIMULATION. Charles H.K. West and Richard P. Michael. Department of Psychiatry, Emory University School of Medicine, and Georgia Mental Health Institute, 1256 Briarcliff Road, Atlanta, GA 30306.

The relation between the midbrain dopamine (DA) systems and reinforcement and unit responses to sensory input suggests that DA might influence responses to sensory cues related to reward. Previously we reported that sensory-evoked unit responses in DA terminal regions were affected by electrical stimulation of DA neurons in the ventral tegmental area (VTA). Here we report that such units respond differentially to auditory stimuli that were and were not paired with reinforcing brain stimulation. Adult male rats implanted with stimulating electrodes in VTA were trained to perform self-stimulation to insure that the stimulus was reinforcing. Then in 3-4 conditioning sessions, animals (n = 8) received 500 msec auditory tone stimuli (CS+) beginning 1 sec before electrical stimulation of VTA. Auditory tones of another frequency (CS-) but equal in number and intensity were randomly administered throughout the sessions. For half the animals, CS+ was 2000 Hz and CS- was 100 Hz, and for the other half the situation was reversed. Subsequently, rats were anesthetized with urethane (1.4 g/kg, ip), and single units were recorded in striatum, nucleus accumbens and olfactory tubercle. Of the 61 units recorded to date, 15 units (25%) responded to auditory stimuli, and 12 of these showed a larger response to the CS+ than to the CS-. Although preliminary, this differential response to auditory stimuli that were or were not cues for reinforcing brain stimulation is consistent with the notion of a reward-related modulation of sensory input by DA.

(Supported by the Georgia Department of Human Resources.)

403.7

DEXMEDETOMIDINE, AN ALPHA-2 ADRENOCEPTOR AGONIST, MODULATES THE ACTIONS OF THE VOLATILE ANESTHETIC ISOFLURANE ON RAT HIPPOCAMPAL SLICE. M.K.T.Savola*, M.B.MacIver, V.A.Doze*, M.Maze and J.J.Kendig (SPON: K.R.Courtney). Department of Anesthesia, Stanford University School of Medicine, Stanford, CA 94305-5117.

Dexmedetomidine (d-med), a highly selective α_2-adrenoceptor agonist, has sedative properties and reduces volatile anesthetic ED_{50}. In order to understand how α_2-adrenoceptor agonists reduce volatile anesthetic requirements, we studied whether d-med modulates isoflurane effects on the electrophysiological responses of the rat hippocampal slice. Synaptically evoked field potentials were recorded from CA1 neurons using standard extracellular techniques. D-med was perfused onto the slice and isoflurane was administered via the surface gas stream. Isoflurane (0.14-1.4 v%) depressed excitatory postsynaptic potentials (EPSP) and population spike responses in a dose-dependent manner. D-med (10 nM-1 μM) itself had no effect on the EPSP, and only small and variable effects on the population spike. Rauwolscine (1-10 μM) was used to antagonize the effects of d-med. D-med enhanced the isoflurane-induced depression of EPSP and population spike amplitude. In addition, the rate of onset of isoflurane effects was increased. Thus, an α_2-adrenoceptor agonist, at a concentration which itself has little effect, potentiates volatile anesthetic depression of output in hippocampal CA1 neurons.

403.8

ALPHA-2 ADRENERGIC MODULATION OF SLOW NOCICEPTIVE AND FAST MONOSYNAPTIC REFLEXES IN NEWBORN RAT SPINAL CORD. J.J.Kendig, M.K.T.Savola* and M.Maze. Dept. of Anesthesia, Stanford Univ. Sch. Med., Stanford, CA 94305-5117

Dexmedetomidine (d-med) is a potent specific α_2 adrenergic receptor agonist with analgesic and sedative properties. To probe spinal α_2 adrenergic effects, the isolated superfused spinal cords of 1-4 day old Sprague-Dawley rats were arranged to stimulate a dorsal lumbar root and record from the corresponding ipsilateral ventral root. Evoked potentials consisted of the low threshold fast (2-50 msec) mono- and polysynaptic reflexes and a high threshold response of very slow time course (to 30 sec) which has been linked to nociception. D-med (1-5 nM) reversibly abolished the slow potential without affecting the monosynaptic reflex. L-medetomidine (l-med) (1 μM) had no effect. Depression of the slow potential was rapidly and completely reversed by the α_2 antagonists atipamezole and rauwolscine. At slightly higher concentrations d-med (10 nM), but not l-med (10 μM), reversibly depressed the monosynaptic reflex. Neither rauwolscine nor atipamezole antagonized depression of the monosynaptic reflex at concentrations at or above those which restored the slow response. The results show a potent α_2 adrenergic inhibition of presumed nociceptive transmission in this preparation. The monosynaptic reflex is also strongly stereospecifically inhibited, but a distinct receptor population appears to be involved.

403.9

MULTIPLE PROJECTION PATHWAYS FROM THE VENTROLATERAL MEDULLA TO LOCUS COERULEUS IN RAT. E.J. Van Bockstaele, B. Astier*, V.A. Pieribone, G. Aston-Jones and M.T. Shipley. Div Behav Neurobiol, Dept Mental Hlth Sci, Hahnemann Univ, Phila., PA 19102;[1]Dept Cell Bio Anat, U Cincinnati, OH 45267.

We have previously found that VLM (nucleus paragigantocellularis) is a major afferent to locus coeruleus (LC). Here, we examined efferent projections from this area using Phaseolus vulgaris-leucoagglutinin (PHA-L). Anterograde labeling was identified in LC as well as many other brain nuclei. We examined the projection from VLM to LC in detail to i) explore possible topography of innervation in LC and ii) describe the pathway(s) taken by VLM fibers projecting to LC.

Multiple iontophoretic deposits of PHA-L were made into VLM and sections were processed using PAP histochemistry. Alternate sections were subjected to fluorescent double labeling with antibodies against PHA-L and phenylethanolamine N-methyltransferase (PNMT), an adrenergic marker. These initial experiments revealed 2 distinct fiber pathways from rostral VLM to LC. Furthermore, these pathways appear to arise from different locations in VLM: caudomedial injections labeled a medial pathway and rostrolateral injections labeled a distinct lateral pathway. Few fibers were seen in the area of the adrenergic medullary bundle (MB), a previously suggested pathway from C1 neurons to LC.

To test the existence of VLM-LC projections through the MB pathway, another set of experiments was conducted with lesions of the MB (rostral medulla level) and 15 days later Fluoro-Gold (FG) was iontophoretically deposited into LC. Sections were processed for PNMT immunofluorescence and examined under UV and rhodamine epi-illumination for FG retrogradely labeled cells and PNMT immunoreactivity, respectively. In initial studies, MB lesions yielded a large (91%) decrease in doubly labeled PNMT/FG neurons and a 56% decrease in FG-only labeled neurons. The failure to label this pathway with PHA-L may reflect a sampling problem or insufficient uptake of PHA-L by PNMT neurons.

These results reveal 3 separate pathways from the VLM to LC. Additional studies are needed to characterize neurons projecting to LC by these pathways. Support: PHS grant NS24698 and ONR/AFOSR Contract N00014-86-K-0493.

403.10

INFLUENCE OF PERIACQUEDUCTAL GREY ON RAT LOCUS COERULEUS NEURONS. [1]M. Ennis, [1]M. Behbehani, [3]E. Van Bockstaele, [3]G. Aston-Jones, and [2]M.T. Shipley., [1]Dept. Physiol & [2]Dept. Anatomy, U. Cincinnati Coll. Med., Cincinnati, OH 45267; [3]Dept Mental Hlth. Sci., Hahnemann Univ. Philadelphia, PA 19102.

Recent anatomic studies reveal that locus coeruleus (LC) receives only few afferent inputs. The afferent status of areas nearby LC has been difficult to ascertain. Here, we have examined possible projections from one nearby area, periacqueductal grey (PAG), recently reported to project to the subcoeruleus (Bietz, 1988).

PHA-L injections in PAG laterally adjacent to the acqueduct labeled fibers that profusely innervate the region rostral and medial to LC, and nucleus parabrachialis. A few fibers appeared to terminate in LC. In agreement with these anatomic results, focal electrical stimulation of LC produced sparse antidromic activation in PAG; 3/34 PAG neurons were antidromically driven at latencies of 4-6 ms (mean threshold = 225 uA). These driven cells did not respond to electrical stimulation of the hindpaw, a manipulation that activates LC neurons.

Stimulation of PAG (.5 Hz) synaptically activated 60% of LC cells tested at onset latencies of 4-10 ms. Such excitation was weak and typically required high currents (>500 uA). Stimulation of PAG lateral to the acqueduct activated 77% of LC neurons usually at shorter latencies (4-7 ms), similar to those for PAG neurons antidromically driven from LC. Stimulation of ventrolateral PAG yielded antidromic or pure inhibitory responses.

These results indicate that PAG may provide sparse innervation of LC. Supported by PHS Grants NS20643 and NS24698.

403.11

DENDRITES OF RAT LOCUS COERULEUS ARE ASSYMMETRICALLY DISTRIBUTED: IMMUNOCYTOCHEMICAL LM AND EM STUDIES. Libang Fu[1]*, M.T. Shipley[1] and G. Aston-Jones[2] (SPON: H. Duncan) Dept Anatomy & Cell Biol. Univ of Cincinnati, Coll. Med. Cinti., OH 45267[1]. Dept. Mental Hlth. Sci., Hahnemann Univ., Philadelphia, PA 19102.

Nissl, Golgi and immunocytochemical (ICC) studies have described the morphology and ultrastructure of rat locus coeruleus (LC). However, much less is known about LC neuronal processes extending outside of the nucleus proper. Here, ICC LM and EM techniques were used to examine the distribution of TH- and DBH-labeled extranuclear processes in the pericoerulear region. DBH-labeled processes preferentailly extended into the rostromedial pericoerulear region. In horizontal sections it is clear that even neurons in the caudal pole of LC preferentially extend their dendrites in a medial and rostral direction and can extend rostrally beyond the rostral pole of the nucleus. Some labeled processes extend caudally but very few were observed in regions lateral to LC.

EM-ICC analysis revealed that of 160 TH- or DBH-labeled profiles identified in the rostral and medial pericoerulear regions all were dendrites. Labeled axons were not observed. These results indicate that the dendrites of LC neurons are very highly oriented. The vast majority of extranuclear LC dendrites extend and ramify in the rostromedial pericoerulear region. Our previous studies demonstrate that the major inputs to LC, from prepositus hypoglossi and paragigantocellularis, terminate in LC proper and also the pericoerulear region medial and rostral to LC. Recent studies demonstrate dense GABAergic and peptidergic terminals in rostral and medial pericoeruleus. Thus, this pericoerulear region may be a key site for regulation of LC. (Supported by PHS Grants NS20643, NS 24698 and NS23348.)

403.12

D_1 AND D_2 DOPAMINE RECEPTOR AGONISTS DECREASE INWARDLY RECTIFYING CURRENTS IN THE CENTRAL NUCLEUS OF THE RAT AMYGDALA. M.C. Schiess and P. Shinnick-Gallagher. Dept. Pharmacology, Univ. Texas Medical Branch, Galveston,TX 77550.

The membrane activity of dopamine (DA) and the specific D_1 agonist SKF 38393 (SKF) and the D_2 agonist Quinpirole (Quin) was studied at a postsynaptic projection site, the central nucleus of the rat amygdala (ACe). Micromolar concentrations were superfused onto 500 micron thick in vitro slice preparations and intracellular membrane activity was recorded under voltage clamp. There are multiple cell types within the ACe that show varying degrees of rectification. The effects of DA, SKF, and Quin are voltage dependent, but not dependent on the degree of rectification within each cell. In 13 cells DA, Quin and SKF produced outward currents at all potentials, creating a parallel shift of the I-V relation. In other cells, DA agonists produced outward currents at holding potentials of -60 and above; no effect from -60 to -90 mV; and from -100 to -160mV either blocked the anomalous rectifier or decreased rectification (N=16). In conclusion, both D_1 and D_2 agonists decrease inwardly rectifying currents. The voltage dependency of DA agonists explains their variable effects in unclamped neurons.

403.13

EFFECTS OF REPEATED COCAINE ADMINISTRATION ON THE SENSITIVITY OF D1 AND D2 RECEPTORS IN THE NUCLEUS ACCUMBENS: ELECTROPHYSIOLOGICAL STUDIES. D.J. Henry and F.J. White. CCN Program, Dept. of Psychiatry, Wayne St. Univ. Sch. of Medicine & Neuropsychopharmacology Lab., Lafayette Clinic, Detroit, MI 48207.

The dopamine (DA) neuronal projection to the nucleus accumbens (NAc) is important in mediating the rewarding effects of cocaine (COC). We have recently reported that NAc neurons are supersensitive to the inhibitory effects of iontophoretic DA following twice daily 10 mg/kg i.p. COC injections for 14 days. The present study will determine the relative contributions of D1 and D2 DA receptors in this sensitization phenomenon, using iontophoresis of selective D1 (SKF 38393) and D2 (quinpirole) agonists. Our preliminary results suggest that NAc neurons from COC-treated rats are inhibited to a greater extent by SKF 38393 as compared to NAc neurons from control rats. Slightly supersensitive inhibitory responses to quinpirole were also observed. Preliminary findings indicate that NAc neurons in COC-treated rats may also be supersensitive to serotonin, another important transmitter involved in mediating the effects of COC within the NAc. (Supported by DA 04093 and MH 40832 to FJW)

403.14

A10 DOPAMINE SOMATODENDRITIC AUTORECEPTOR SENSITIVITY FOLLOWING WITHDRAWAL FROM REPEATED COCAINE TREATMENT. J.M. Ackerman and F.J. White. Neuropsychopharmacol. Lab., Dept. Psychiat., CCN Program, Wayne St. Univ. Sch. Med., Lafayette Clinic, Detroit, MI 48207.

We have previously demonstrated that impulse-regulating autoreceptors on A10 dopamine (DA) cells in the rat ventral tegmental area become subsensitive to DA and DA agonists following repeated administration of cocaine. The present study sought to determine if this subsensitivity persists following cocaine withdrawal. Male rats were given twice daily i.p. injections of 10 mg/kg cocaine or saline for 14 days. Three groups were then tested at various times after cocaine withdrawal: 1, 4 or 7 days. Extracellular single-unit recording techniques were used to determine the sensitivity of somatodendritic autoreceptors on A10 DA cells as indicated by the inhibitory effects produced by i.v. apomorphine (APO). As previously reported, A10 DA neurons were subsensitive to APO 1 day after the last injection of cocaine. Following four days of withdrawal, subsensitive responses to APO were still observed, although the extent of this effect was reduced as compared to rats tested 1 day after the last injection. After seven days of withdrawal, the sensitivity of DA autoreceptors had recovered to the level of saline control animals. Thus, autoreceptor subsensitivity produced by repeated cocaine is a transient alteration that cannot account for long-lasting sensitization, although it may be necessary to trigger other permanent changes (Supported by DA-04093 and MH-40832).

403.15

MORPHINE WITHDRAWAL ACTIVATION OF LOCUS COERULEUS NEURONS: ATTENUATION BY LESIONS OF NUCLEUS PARAGIGANTOCELLULARIS. Kurt Rasmussen and George K. Aghajanian. Depts. of Psychiatry and Pharmacology, Yale Univ. Sch. Med., New Haven, CT 06508.

The locus coeruleus (LC) contains the densest aggregate of norepinephrine containing neurons in the rat brain. In anesthetized rats LC neurons are inhibited by systemic or local administration of opiates. Tolerance to the inhibitory effects of opiates develops in the LC with chronic administration and these neurons display a strong naloxone-induced withdrawal activation. However, LC neurons do not show withdrawal induced activation in vitro in brain slices taken from morphine dependent rats, indicating that this activation is mediated by afferents to the LC. Two major afferents to the LC were lesioned in an effort to block the withdrawal-induced activation. Morphine pellets (75 mg) were implanted subcutaneously daily for two days. Single unit recordings were made two days after the last morphine pellet was implanted. Withdrawal was induced by administering naltrexone HCl (10 mg/kg) subcutaneously. Radio-frequency lesions were placed in either the nucleus paragigantocellularis (PGi; unilateral) or prepositus hypoglossi (PrH; bilateral). Lesions of the PrH did not prevent the withdrawal-induced activation of LC neurons, while lesions of the PGi greatly attenuated (but did not completely block) the withdrawal-induced activation. In PGi lesioned animals, the withdrawal-induced activation was attenuated in the LC ipsilateral to the PGi lesion, but was not attenuated in the LC contralateral to the lesion. Since the excitatory projection from the PGi to the LC has been shown to employ an excitatory amino acid (Ennis and Aston-Jones, J. Neurosci. 8 (1988) 3644), some animals were pretreated with kynurenic acid (an excitatory amino acid antagonist). Kynurenic acid (1 µmol in 10 µl, i.c.v.) completely blocked the withdrawal-induced activation of LC neurons. These studies indicate that the morphine-withdrawal-induced activation of the LC is mediated at least in part by an excitatory amino acid projection from the PGi.

403.16

D-1 SPECIFIC AGENTS MODULATE DOPAMINERGIC TERMINAL EXCITABILITY: FURTHER STUDIES. C. Okuda, M. Diana , S. Young & P.M. Groves. (SPON. J.C. Linder)Dept. of Psychiatry, Univ. of California San Diego, La Jolla, CA 92093.

Previous evidence has shown that striatal infusion of a D1 specific agonist, R-SKF 38393, produces a decrease in the electrical excitability of dopaminergic nigrostriatal terminals which is reversed by subsequent infusion of SCH 23390, a D1 specific antagonist. These studies suggest that D1 receptors are present on these terminals. In order to provide further evidence for this possibility, we have used two new benzazepine molecules, SKF 77434 (D1 agonist) and SKF 83566 (D1 antagonist), which have been recently introduced as D1 selective agents, (O'Boyle and Waddington, 1987). An infusion cannula and an adjacent bipolar stimulating electrode were implanted in the neostriatum of urethane anesthetized rats. Terminal excitability of nigrostriatal dopaminergic neurons was assessed before and after drug infusions. Striatal infusion (300nl, 5 mins) of SKF 77434 (1 µM, 10 µM) produced a dose related decrease in terminal excitability. SKF 83566 (1µM, 10 µM) also decreased excitability. Although the inhibitory action of the antagonist SKF 83566 appears paradoxical, it is consistent with the effect of another antagonist, SCH 23390, observed previously. Experiments involving reversal of the action of SKF 77434 by SKF 83566 will also be presented. These results provide further evidence for the presence of D1 receptors on dopaminergic axon terminals in the neostriatum. (Supported by NIDA.)

403.17

RESPONSES OF SOMATOSENSORY CORTICAL NEURONS TO IONTOPHORETICALLY APPLIED MONOAMINES IN THE UNANESTHETIZED RAT. M.H. Bassant, K. Ennouri and Y. Lamour, INSERM, U. 161, 75014 Paris, France.

Experiments were performed on unanesthetized rats using a painless restraint system fixed on the skull. Electrode consisted of a recording micropipette (1M NaCl, 2% pontamine blue) attached to a multibarreled micropipette for iontophoresis. Electrode penetrations were reconstructed on camera lucida drawings of brain sections. The effects of 5-hydroxytryptamine (5-HT), dopamine (DA) and noradrenaline (NA) were studied in 130 neurons of the S-I cortex. The 3 amines had predominantly depressant effects. A comparison of the percentages of neurons inhibited revealed that NA was the most potent (80% of neurons inhibited) followed by 5-HT (73%) and DA (67%). When the amines were applied with equivalent current to the same neuron, the effect of NA was also the strongest. The laminar distribution of the responses showed that the neurons of the infragranular layers were clearly more sensitive to 5-HT and DA, compared to superficial layers while the responses to NA were distributed more uniformly, with the highest percentage of neurons inhibited in layer VI. After NA or 5-HT applications, the acetylcholine-induced excitation was suppressed or decreased in 60% of the cells tested. These results provide evidence of widespread effects of monoamines on S-I neurons in awake rats, but also of laminar specificity.

Supported by Bayer Pharma France.

403.18

THE FIRING RATE OF VENTRAL PALLIDAL NEURONS IS AFFECTED BY DOPAMINE AGONISTS. R.J. Maslowski* and T.C. Napier, (SPON: A. Karczmar). Dept. Pharmacol., Loyola Univ. Stritch Sch. Med., Maywood Il 60153.

The ventral pallidum (VP) receives monosynaptic inputs from a dopaminoceptive region, the ventral striatum. Thus, activation of dopamine receptors in the ventral striatum may also affect VP neuronal activity. The activity of VP neurons was assessed following systemic injection of apomorphine, a nonspecific dopamine agonist. The firing rate of 82% of the VP neurons tested (n=34) was affected by apomorphine (0.5mg/kg,iv) and the responses were equally distributed between increases and decreases. The D1 antagonist, SCH23390 (0.1mg/kg,iv) blocked 70% of the apomorphine-induced responses (n=16). Sulpiride (12.5mg/kg,iv), a D2 antagonist, reversed the responses in 50% of the cells (n=12). Subsequently, the effects of the D1 agonist, SKF38393 (0.1-25.6mg/kg,iv), or a D2 agonist, quinpirole (0.025-25.6mg/kg,iv), on VP neurons were examined. Cumulative dose-response curves were generated for each drug. Of the cells tested (n=26), 62% responded to SKF38393 with dose-dependent increases in firing rate (ED_{50}=0.9mg/kg and Vmax=3.2mg/kg). SCH23390 reversed the increase in 88% of the cells. Effects of quinpirole were variable and often insensitive to sulpiride. Thus, the dose-dependence and specific antagonism of D1-mediated responses indicate that the firing rate of VP neurons is sensitive to alteration in D1 receptor activity.

403.19

Subpopulations of Ventral Pallidal Neurons Respond Differentially to Microiontophoretically Applied Dopamine. T.C. Napier. Dept of Pharmacol, Loyola Univ of Chicago, Maywood, IL 60153.

The response of ventral pallidal (VP) neurons to microiontophoretically applied dopamine (DA) was studied in chloral hydrate anesthetized rats to determine if subpopulations respond differentially to this neurotransmitter. To characterize neuronal subpopulations, the following were recorded: histological placement, firing rate (Hz), firing pattern, action potential amplitude and duration, wave form (monophasic, biphasic or triphasic), and the direction of initial deflection of the action potential (negative or positive).

Forty-two percent of 101 VP neurons responded to DA (0.2M, 5-60nA); firing rates were decreased in 29 cells and increased in 13. Computer-generated cross tabulations revealed that, as compared to non-responders, cells which were suppressed by DA had a slower firing rate ($18.8^{\pm}1.9$ vs $11.6^{\pm}1.6$ Hz). Additionally, these cells had action potentials which were smaller ($716^{\pm}317$ vs $92^{\pm}46$ uV) and biphasic (61% vs 72%) with a negative initial deflection (53% vs 97%). Cells which were excited by DA did not differ from non-responders. These data suggest that a distinct subpopulation of VP neurons can be identified, based upon certain electrophysiological characteristics and their responses to DA.

Work supported by BSRG, Loyola University of Chicago.

403.20

ALTERATIONS IN CONCENTRATIONS OF 3-METHOXY-4-HYDROXYPHENYLETHYLENEGLYCOL (MHPG) IN THE PARAVENTRICULAR NUCLEUS (PVN) AND SUPRAOPTIC NUCLEUS (SON) REFLECT THE ACTIVITY OF NORADRENERGIC (NE) NEURONS PROJECTING TO THESE HYPOTHALMIC REGIONS. K.J. Lookingland, L. Ireland*, Y. Tian*, J. Manzanares and K.E. Moore. Dept. of Pharmacol/Toxicol, Mich. State Univ., East Lansing, MI 48824

In the rat brain MHPG, a major metabolite of NE, exists predominantly as a conjugate and, as such, cannot be quantified using HPLC coupled to a electrochemical detector. In the present study, an acid hydrolysis procedure for deconjugating MHPG was developed and utilized to determine MHPG concentrations in the PVN and SON following procedures that activate NE neurons. MHPG was not detectable in untreated samples, but following acid hydrolysis significant amounts of MHPG were measured in the PVN and SON. Activation of NE neurons by physical restraint or the administration of the α_2-adrenoceptor antagonist idazoxan increased MHPG concentrations in both the PVN and SON. On the other hand, electrical stimulation of the locus coeruleus increased MHPG concentrations in the PVN, but not in the SON, which is predominantly innervated by NE neurons originating in sub-coeruleus regions of the pons-medulla. These results indicate that procedures that activate NE neurons increase MHPG concentrations in the PVN and SON. The concentration of this metabolite should be a useful index of the activity of NE neurons projecting to these hypothalamic nuclei. (Supported by NIH grant NS24113.)

403.21

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

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

DIFFERENTIATION. MORPHOGENESIS AND DEVELOPMENT: TRANSMITTERS AND ENZYMES

404.1

ELECTRON MICROSCOPIC IDENTIFICATION OF LUTEINIZING HORMONE-RELEASING HORMONE (LHRH) IMMUNOREACTIVITY IN OLFACTORY PLACODE-DERIVED NEURONS L.M.Zheng, D.W.Pfaff and M.Schwanzel-Fukuda. Rockefeller University, New York, New York.

LHRH neurons originate in the olfactory placode and migrate into the forebrain along branches of the terminalis and vomeronasal nerves (Nature,1989, 338:161-164). The present study examined, in mouse, the ultrastructure of these LHRH-ir neurons during migration and found that the subcellular location and density of LHRH-ir product varied with the location of the LHRH-ir cells along the migratory route. LHRH-ir neurons, first detected at E 11 in the epithelium of the medial part of the olfactory placode just before migration, showed LHRH-ir product heavily accumulated around the outer nuclear envelope and in the lumen of the rough endoplasmic reticulum (rER) adjacent to the nuclei. From E 11.5 through E 14, large numbers of LHRH-ir cells were seen in cords on the nasal septum, with LHRH-ir product distributed throughout their cytoplasm as well as around their nuclei and in the lumen of the rER. Neither Golgi apparatus nor LHRH neurosecretory granules were seen in the LHRH neurons from E 11 to E 16, and no synapses were found on these cells. By E 18, LHRH neurons were seen in the forebrain and LHRH-ir beaded neurites formed dense plexuses in the OVLT and in the ME.
Supported by NIH grant 19662 and the Whitehall Foundation.

404.2

NEONATAL GONADECTOMY HAS GENOTYPE DEPENDENT EFFECT ON BRAIN DOPAMINE SYSTEMS AND BEHAVIOR.
P. Kabai*, I. Sziraki*, A. Lajtha and C. Vadasz. Div. Neurochemistry, The Nathan S. Kline Institute, Orangeburg, N.Y. 10962.

Recent studies demonstrated, that perinatal antiandrogene treatment lowered Tyrosine hydroxilase (TH) activity in dopaminergic brain areas in a strain dependent manner (Vadasz, Cs. et al., Hormones and Behav., 22:528, 1988). Here we wished to address the question whether this "critical period" is restricted to prenatal development, or neonatal orchiectomy would also have strain dependent organizational effects on brain dopamine systems.

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

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

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

404.3

MORPHOMETRIC ANALYSIS OF CULTURED HYPOTHALAMIC TYROSINE HYDROXYLASE AND NEUROPHYSIN CELLS. M. Morris*, B.A. Bennett, J. Clodfelter*, D.K. Sundberg*, Department of Physiology and Pharmacology, Wake Forest University Medical Center, Winston-Salem, NC 27103.

Studies were performed to characterize the effect of a depolarizing KCl stimulus on specific hypothalamic cell types. Hypothalamic cultures were stained immunochemically for neuron specific enolase (NSE), neurophysin (NP) or tyrosine hydroxylase (TH) and neuronal density and size were quantitated. The cultures were prepared from neonatal hypothalami which were enzymatically dispersed. Control or KCl treated (25 mM) cultures were fixed after 7 days and stained for NSE, NP or TH. KCl caused a universal increase in cell size. The cell area was increased from 99.4 and 123 mm^2 in the controls to 140.3 and 167.7 mm^2 in the KCl cultures (NP and TH, respectively). A change in the pattern of NP cell size was also noted. The KCl treatment resulted in a greater proportion of large NP positive cells (144.2 vs.84.5 um^2). With regard to cell density, the most marked effect was on the NSE population. The cell density was 117.9/mm^2 as compared to 223.4/mm^2 in the KCl treated cutures. There was also an increase (29%) in the number of NP positive cells, while the density of TH cells was not altered. This morphometric study illustrates that treatment with a depolarizing stimulus has specific effects on cultured hypothalamic neurons. It enhances survival of the total neuron population as well as increasing the size of TH, NP and NSE cells.

404.4

DEVELOPMENT OF TYROSINE HYDROXYLASE (TH)-IMMUNOREACTIVE NEURONS IN THE CHICK EMBRYONIC BRAIN.
J.A. Wallace, A.J. Vallejos*, A.A. Romero*, P.C. Allgood*, and I. Lopez-Colberg. Dept. of Anatomy, Univ. of New Mexico Sch. of Med., Albuquerque, NM 87131.

The development of catecholaminergic (CA) neurons in the embryonic chick brain has been investigated previously by fluorescent histochemical methods. Here, we have compared the results of these earlier studies with an examination of the ontogeny of CA neurons investigated by anti-TH immunocytochemistry. TH-containing cells were demonstrated by the peroxidase ABC method of staining paraffin sections. Embryos were examined at intervals of 5,7,9,15 and 21 (E21) days of incubation. The earliest immunoreactive cells were found at E5. Numerous immature TH-immunopositive neurons were present in rostral portions of the lateral hypothalamus. By E7, at least 3 distinct groups of deeply-stained cells were observed throughout the hypothalamus, while also staining was detected in a small number of cells within the region of the developing substantia nigra. With further progression, the majority of CA cell groups defined histochemically in the adult chicken were recognizable in the E9 embryo. By E15, the mature distribution and number of cells in the various brainstem CA nuclei were clearly evident. The results of our immunocytochemical study demonstrate the presence of TH in neurons of the early chick embryo brain that far precedes the earliest histochemical detection of CA in these cells. Overall, though, the differentiation of CA nuclei appears to lag far behind that of central serotonergic systems.
Funded by NIH grants RR-08139 and BSRG award SO7-RR05583-24.

404.5

SEROTONIN AND MORPHOGENESIS DURING NEURULATION AND CRANIOFACIAL DEVELOPMENT IN THE MOUSE. D.L. Shuey*#, R. Thomas*, M. Yavarone*, H. Tamir, T. Sadler* and J. Lauder. Dept. of Cell Biology and Anatomy and #Curr. in Toxicology, Univ. of North Carolina Sch. of Med., Chapel Hill, NC 27599.

We have previously identified numerous uptake sites for serotonin (5-HT) in epithelial structures of the developing craniofacial region of the gestational day (GD) 12 mouse embryo, and serotonin binding protein (SBP) in adjacent mesenchyme, suggesting a possible role for 5-HT in epithelial-mesenchymal interactions (Lauder et al., Develop. 102:709, 1988).In the present study, GD 9-12 mouse embryos were cultured in the presence of 5-HT or one of its precursors. In some cases, an MAO inhibitor and/or 5-HT uptake inhibitor were added. Sites of 5-HT uptake or synthesis and SBP localization were then identified using immunocytochemistry. 5-HT immunoreactivity (IR) was seen in the heart, visceral yolk sac, gut, thyroid, limb-bud epithelium, eye primordium and specific regions of the neural epithelium, in addition to the sites previously identified at GD 12. Only the heart and yolk sac seemed to be sites of 5-HT synthesis. At GD 9, SBP IR appeared throughout the mesenchyme but became more localized in the older embryos adjacent to epithelial sites of 5-HT uptake. 5-HT IR was also found in the ectoplacental cone at GD 9, and in the placenta of older embryos suggesting that the maternal embryonic blood supply may provide a source of 5-HT in vivo.

404.6

HELISOMA EMBRYOGENESIS: MORPHOLOGICAL, BEHAVIORAL AND NEURAL DEVELOPMENT. K. McKenney and J.I. Goldberg. Dept. of Zoology, Univ. of Alberta, Edmonton, T6G 2E9.

Embryos of the fresh water snail, Helisoma trivolvis, are accessible to developmental and behavioral studies throughout embryogenesis. In vivo and in vitro studies have established this as a useful model system for comparing neuronal development with adult plasticity and regeneration. The objectives of this study were to quantitatively describe the stages of embryogenesis and the temporal sequence of gangliogenesis. Morphological (scanning electron microscopy) and behavioral criteria were used to define embryo stages, which were expressed as a percentage of the duration of embryogenesis. Gangliogenesis was analysed using light microscopic histological techniques. Cilia-driven spinning, the first recognizable behavior, began at 20% of embryonic development (stage E20). Other embryonic behaviors and their onset were cardiac beating, body wall contractions (both at E45), ciliary crawling (E50) and coordinated buccal rasping (E70). Gangliogenesis also began at E20, with the formation of the cerebral ganglia. Next to form was the pedal ganglia, at E25, followed by the buccal ganglia (E30), the pleural ganglia (E35) and finally, the parietal/visceral ganglia at E40. Morphologically, the characteristic veliger and juvenile forms appeared at E30 and E50, respectively.

Funded by NSERC of Canada and AHFMR.

404.7

EFFECTS OF SEROTONIN ON INTRACELLULAR CALCIUM IN EMBRYONIC AND MATURE *HELISOMA* NEURONS. J. I. Goldberg, L. R. Mills and S. B. Kater. Dept. of Zoology, University of Alberta, Edmonton, Alberta, T6G 2E9 and Program in Neuronal Growth and Development, Colorado State University, Fort Collins, CO 80526.

In cultures of dissociated embryonic or adult *Helisoma* neurons, serotonin either stimulates or inhibits outgrowth in a large portion of the neurite population. Since calcium has been implicated in the regulation of neurite outgrowth in an identified neuron, we now examine intracellular calcium in dissociated embryonic and mature neurons using the Fura2 method. The main goals of this study are to compare calcium levels in developing and regenerating neurons, and to determine how serotonin influences intracellular calcium concentration throughout the neuronal population. In embryonic neurons, calcium levels declined significantly as a function of days in culture (day 3: 120.1 ± 9.8 nM, N=15; day 11: 68.9 ± 6.4 nM, N=49). In regenerating adult neurons, calcium levels were characteristically higher (149.4 ± 12.5 nM, N=110), and extended over a greater range than in the embryonic neurons. In both embryonic and adult cultures, the mean calcium concentration was increased after addition of 50 µM serotonin. When examined on an individual neuron basis, serotonin increased the calcium concentration in approximately 50% of the neurons. Thus, although the effect of serotonin on neurite outgrowth can be stimulation or inhibition, its effect on intracellular calcium is primarily stimulation.

Supported by NSERC of Canada, AHFMR and NIH.

404.8

DEVELOPMENT OF NEUROPEPTIDE EXPRESSION IN SYMPATHETIC GANGLIA OF THE BULLFROG TADPOLE; EVIDENCE FOR SPECIFICATION BY THE PERIPHERY. W.D. Stofer and J. P. Horn Dept. of Physiology, University of Pittsburgh School of Medicine, Pittsburgh, PA 15261.

These experiments determined the expression of immunoreactivities (-IR) for tyrosine hydroxylase (TH), 150 kD neurofilaments (NF), neuropeptide Y (NPY), leuteinizing hormone releasing hormone (LHRH), calcitonin gene-related peptide (CGRP) and substance P (SP) in the developing bullfrog sympathetic system. Staged tadpoles were perfused with 4% paraformaldehyde, and sections containing paravertebral ganglia 9 and 10 and the hindlimbs were processed for indirect immunofluorescence.

By stage III, axons containing NF-IR are already present in ganglia 9 and 10. Subsets of the axons contain LHRH-IR, CGRP-IR, and SP-IR. However, these fibers do not appear to make synaptic contacts with most postganglionic neurons based on their sparsity and the absence of synaptic boutons. At stage III, TH-IR is present only in a few postganglionic neurons. NPY-IR first appears in postganglionic neurons at stage XI. Some NPY-positive cells are TH-negative at stage XII. Subsequently, the proportions of neurons expressing TH-IR and NPY-IR gradually increase. Synaptic boutons and the adult pattern of NPY-IR appear after stage XVIII. Formation of functional nicotinic synapses in the ganglia (Marshall, Soc.Neurosci.Abst.8: 866,1982) thus occurs at least 15 stages after the arrival of preganglionic and sensory axons.

Tadpole stages are largely defined by the emergence and development of the hindlimbs which are the primary targets of neurons in sympathetic ganglia 9 and 10. We therefore examined developing hindlimbs for TH-IR and NPY-IR. Between stages IV-XI, TH-positive axons are found primarily within bundles in the mesenchyme. By stage XII, well before the appearance of bouton-like structures in the ganglia, TH- and NPY-positive axons are present around subdermal blood vessels. The adult pattern of TH and NPY staining in skin begins to emerge around stage XV in concert with differentiation of the skin glands. Together, our results suggest that postganglionic neurons contact their peripheral targets before being innervated by preganglionic axons and that ganglionic synapse formation and expression of NPY-IR may depend upon the differentiation of peripheral targets. Supported by NIH grant NS21065.

404.9

EXPRESSION OF NEUROACTIVE SUBSTANCES IN THE DEVELOPING CHICK SYMPATHO-ADRENAL SYSTEM. J.E.García-Arrarás, A.M. Lugo*, L.Medina* and R.Martínez. Dept. Biology, Univ. of Puerto Rico, Río Piedras, Puerto Rico 00931.

In the sympatho-adrenal system subpopulation of cells express different neuroactive substances. We have used immunocytochemistry and radioimmunoassays (RIA) to study the expression of immunoreactivity similar to met-enkephalin (M-ENK), neuropeptide Y(NPY) and serotonin (5HT) in the adrenal and in the paravertebral ganglia of the chicken throughout development. Immunoreactivities to the two neuropeptides and the monoamine are expressed from very early in both the adrenal and sympathetic ganglia primordia. The same cells are known to express catecholamines and somatostatin raising the possibility that some cells might be expressing five neuroactive substances at this early developmental stage. However, their pattern of expression differs. 5HT and NPY seem to be present in all chromaffin cells while M-ENK is preferentially expressed by a subpopulation. RIA shows that the amount of NPY/protein increases during embryonic and posthatching development reaching a maximal value in the adult. M-ENK/protein values decrease during development, with a maximal value early in sympathoadrenal differentiation. In the sympathetic ganglia M-ENK and 5HT are expressed transiently. NPY, on the other hand, is expressed all throughout development by about 30% of the neuronal population. The amount of NPY increases during embryonic development reaching its maximal value before eclosion.

Our results show that each neuroactive substance has a particular expression pattern in the developing sympathoadrenal system, suggesting that the phenotypic expression and development of each neuropeptide and monoamine are controlled by specific factors. (Supported by NIH-RR-08102, NSF-BNS-8801538 and by the FIPI program of the U.P.R.

404.10

DEVELOPMENT OF VASOACTIVE INTESTINAL PEPTIDE IMMUNOREACTIVITY IN NEURONS IN THE CHICK PROVENTRICULUS. Miles L. Epstein. Dept of Anatomy, University of Wisconsin, Madison, WI 53706.

Enteric neurons develop from neural crest cells which migrate, proliferate, and differentiate within the gut. Factors that regulate the number and the transmitter phenotypes of the enteric neurons are unknown. We have carried out studies to describe the development of neurons in enteric ganglia in different regions of the developing chick gut, with the aim of pinpointing the time when critical developmental decisions are made. Gut from embryos, hatchlings, and 4-5 week old chicks was fixed and immunostained with antisera to VIP. Tissue from hatchlings and 4-5 week old animals was removed, incubated in organs baths with media containing colchicine, fixed, and immunostained. Whole mounts of myenteric ganglia from the chick proventriculus (secretory portion of the avian stomach) were prepared and the number of VIP-stained cells in a ganglion was counted. At 6.5 days of incubation (d.i.), small clusters of VIP neurons (1 or 2) are found throughout the proventriculus with more VIP cells at the proventriculus-gizzard junction than at the proventriculus-esophageal junction. Fiber connectives between the ganglia became prominent at 7.5-8.5 d.i. The number of VIP neurons per ganglion peaks at 15.5 d.i. A large number of VIP cells (approximately 2/3 of the peak value) was observed in ganglia from 4-5 week old animals incubated in colchicine. This information will be useful in describing when decisions about neuron number and transmitter phenotypes are made. (Supported by NSF BNS 8820658 and NIH 32978.)

404.11

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

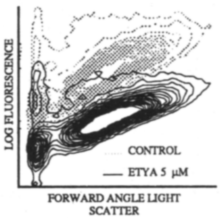
Catecholamine fluorescence (FIF) in clonal cultures of quail neural crest cells is suppressed by norepinephrine uptake inhibitors (Sieber-Blum & Silverman, Neurosci. Abstr. 11, 808, 1985). We here report that transport of tritiated NE was developmentally regulated and that the inhibitory effect was reduced when the addition of DMI (10uM) was delayed, indicating that the cells were most sensitive during the early stages of their in vitro development. Exposure to DMI for 3-day time blocks at various stages of development did not significanty decrease the number of colonies containing FIF-positive cells ($p > 0.57$). However, continuous exposure to DMI caused a pronounced decrease in the number of tyrosine hydroxylase ($p=0.0001$) and dopamine-β-hydroxylase ($p=0.0006$) immunoreactive cells. These data suggest that a) DMI acts before overt expression of the adrenergic phenotype, b) DMI is nontoxic, c) a chronic exposure is necessary, and d) the drug alters catecholaminergic enzyme levels. We conclude that the NE uptake system may play a role in adrenergic development, and that tricyclic antidepressants may influence adrenergic neurogenesis during embryonic development. Supported by USPHS grant HD21423.

BIOCHEMICAL AND PHARMACOLOGICAL CORRELATES OF DEVELOPMENT II

405.1

AN INHIBITOR OF ARACHIDONIC ACID METABOLISM, ETYA, DECREASES OXONOL FLUORESCENCE IN ACUTELY DISSOCIATED VERTEBRATE SPINAL CORD CELLS K.S. Madden*, A. Prasad*, S.V. Smith*, A.E. Schaffner and J.L. Barker. (Spon. R. Nelson) Lab. of Neurophysiology, NINDS, NIH, Bethesda, MD 20892

The function of essential fatty acid (EFA) metabolism in cells is often probed using drugs that reduce the release of eicosanoids from intact cells. We studied the effects of four of these drugs (indomethacin, BW755C, SKF525A or ETYA; 1 µM-1 mM) on cell populations (ca. 20,000/trial) from the embryonic chick (E4.5-8) or rat (E15-18) using flow cytometry and oxonol, a voltage-sensitive dye. ETYA, the acetylenic analogue of arachidonic acid (AA; 5,8,11,14-eicosatetraenoic acid), was the only drug to produce a highly consistent effect. As shown, the drug decreased the signal intensity of most cells, a dose-dependent effect (range: 1-15 µM). ETYA acted on cells depolarized by the cationophore gramicidin, but not when cells were polarized near the potassium equilibrium potential. ETYA seems to increase the potassium conductance of the dissociated cells (neurons, neuroblasts and glioblasts) but the mechanism is uncertain. Each drug used is known to block one or more of the oxygenase pathways that convert AA and certain other EFAs to eicosanoids. ETYA may polarize the embryonic cells by some action unrelated to oxygenase inhibition.



405.2

THE PRENATAL EFFECTS OF SKF 38393, A D-1 AGONIST, ON THE BIOCHEMISTRY AND BEHAVIOR OF NEONATAL RATS. A. Shemer and P.M. Whitaker-Azmitia. Dept. of Psychiatry, SUNY at Stony Brook, NY 11794.

We have developed an in vivo model to assess the role of neurotransmitters during development. In our present study we administered the D1 agonist SKF 38393 1mg/kg to pregnant Sprague Dawley rats from D12 of gestation to parturition. The resultant offspring showed lasting changes in the high affinity uptake of 3H-5HT, indicative of terminal density and in the binding to spiroperidol. On PND 15 the treated neonates were less active in the open field and alternated less than their controls in a spontaneous alternation paradigm. At 90 days neonates were trained in a fixed ratio operant task and, when challenged with serotonergic drugs and dopaminergic drugs, showed significantly less sensitivity to both classes of drugs in a dose dependant manner. The presence of a dopaminergic agonist during gestation produced changes in both the serotonin and the dopamine system which indicates an interaction between the two systems during development, causing a compensatory increase in the outgrowth of the unaffected transmitter system.

405.3

THYRIOD HORMONES ALTER THE POSTNATAL DEVELOPMENT OF THE 68 KD NEUROFILAMENT PROTEIN IN THE CEREBRAL CORTEX: AN IMMUNOCYTOCHEMICAL ANALYSIS. Nancy J. Woolf, Justin D. Oh*, and Larry L. Butcher. Laboratory of Chemical Neuroanatomy, Dept. of Psychology, University of California, Los Angeles, CA 90024-1563, U.S.A.

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

405.4

POST-MITOTIC NEUROMUSCULAR DIFFERENTIATION OF CREATINE KINASE IN POST-NATAL DEVELOPING RAT. O.C. Ramírez, G. Licea* and E. Jiménez*, Depto. de Bioquímica, Centro de Investigación y de Estudios Avanzados-I.P.N., México, D.F. 07000.

Shainberg *et al* (Dev.Biol.25:1,1971) reported 9.20,4.80, 2.27 and 2.20 creatine kinase (CK) U/mg prot. in rat extracts of white muscle, brain, red and heart muscles respectively, whereas liver, kidney and spleen extracts contained only about 0.06 U/mg prot. Irrespective of its blastodermic origin, white muscle and brain contained 4 and 2 times as much CK as either red or heart muscles. Such values could be used as post-mitotic tissue-specific molecular markers. However, we found that the differential expression of CK, in brain and heart only, is regulated in such a way that both tissues showed, almost in parallel, similar values of cytosolic CK specific activity along rat post-natal development and ageing. In contrast to the previous report, skeletal muscle CK values (at least 3 times higher than those of brain and heart) coincided only in morphology of CK developmental patterns. Skeletal muscle to heart-and to brain-CK specific activity ratios had always values greater than 4, except at 370 days, which was only about 2. Contrarily to the established view, it was found that "mature" heart and brain tissues synthesize the BB-CK and MM-CK isoenzyme type, besides their typical "tissue-specific" CK dimers. Shifts in peaks of CK activity and electrophoretic migration distance of isoenzymes seem to reflect differential tissue-specific requirements of ATP formation, that cannot be entirely covered by anaerobic glycolisis and the Krebs cycle.

405.5

THE RAT PUP ULTRASONIC ISOLATION CALL: STUDIES OF SEROTONERGIC AND ADRENERGIC NEUROTOXINS. I.T. Winslow and T.R. Insel. Lab. Clin. Sci., NIMH, NIHAC, Poolesville, MD. 20837.

Rat pups removed from their nest and siblings emit a highly stereotyped and well characterized isolation call in the ultrasonic range. This behavior is selectively sensitive to the effects of benzodiazepines, opiate agonists and antagonists, and monoamine reuptake inhibitors. To further characterize the role of serotonin and noradrenergic transmission in the postnatal development of social behavior, selected doses of MDMA and DSP-4 were administered subcutaneously to pups beginning 24 hr after birth. 10 mg/kg MDMA was administered once or twice daily, for 4 days. 50 mg/kg DSP-4 was administered once on day 1. To balance the experimental treatment design, both DSP-4 and vehicle control pups were injected with saline twice daily for 4 days, and pups in all treatment groups were tested 6, 9, 12, and 15 days postnatally. Body weight and core temperature of pups receiving the high MDMA dose were significantly reduced compared to DSP-4, and saline treated pups. The isolation call of MDMA, but not DSP-4 treated pups was reduced 40-80% depending on the total dose of MDMA received. The frequency of cell crossing and a measure of geotaxis were both unaffected by neurotoxins. Curiously, the effects of monoamine uptake inhibitors on vocal behavior (Winslow and Insel 1989) were not blocked in MDMA and DSP-4 treated pups when expressed as % pretreatment rate. Preliminary data indicate that early postnatal administration of MDMA is associated with a at least a 40% decrease in the number of cortical 5HT uptake sites indicating loss of 5HT terminals (as measured by ^{3}H-paroxetine binding), as well as decreased 5HT and 5HIAA content (as determined by HPLC). In additional experiments we are studying the effects of prenatally administered MDMA on the behavior of both pups and dams. Our data suggest an important role for serotonin in the maintenance of mother-infant attachment behaviors.

405.6

EARLY DEVELOPMENT OF THE CHOLINERGIC SYNAPSE IN RAT AND HUMAN DRAIN. A. Biegon, O. Bar-Peled* and M. Segal*. Weizmann Institute of Science, Rehovot, Israel.

The ontogeny of Acetylcholinesterase (AChE), muscarinic and nicotinic cholinergic receptors was investigated by quantitative histochemistry (Biegon, A. and Wolff M., J. Neurosci. Methods 16:37, 1986) and autoradiography (Biegon et al., J. Neurochem. 51:1381, 1988) in rats from day 16 of fetal life and in human abortuses (16, 18 and 24 weeks gestation). Each marker exhibits a unique and region-specific developmental pattern. Rat AChE activity increases very slowly in cortex, striatum and hippocampus and is still very low compared to adult levels on postnatal (PN) day 21. Cholinergic (e.g. medial septum) nuclei reach adult levels much earlier, between 7 and 14 days PN. In the human fetus, too, adult AChE levels are found in the substantia innominata by the 24th gestational week, while cortical, hippocampal and striatal activity is very low. Nicotinic (α-bungarotoxin) binding is very high at the earliest ages tested in rat and human hippocampus, followed by a decline towards adult levels. Muscarinic receptors in both species increase monotonously with age in most regions, reaching adult or higher levels by day 14 PN in rats and week 24 in humans. High receptor levels preceding the appearance of a presynaptic marker suggest the receptors may play a developmental role in the cholinergic synapse.

405.7

RETINOID REGULATION OF GENE EXPRESSION IN THE EARLY NERVOUS SYSTEM. S-C. Chen*, I. Lugo, A.K. Hall*, J. Hempstead*, R.Ziai* and J.I. Morgan*(SPON: R. Wurzburger). Dept. of Neuroscience, Roche Institute. of Molecular Biology, Roche Research Center, Nutley,NJ 07110.

Using HPLC techniques we have identified polypeptides whose expression; (a) alters during normal neuroembryogenesis and (b) are regulated by retinoic acid in neural cell cultures. Several candidate molecules have been isolated, sequenced and their mRNAs and genes cloned by recombinant DNA techniques. A 43 amino acid polypeptide, termed thymosin beta-10, was found to be very abundant in the early rodent nervous system but both the protein and mRNA disappeared shortly after birth. In several neuroblastoma cell lines, including B104, retinoic acid causes a marked increase in beta-10 protein and mRNA. These particular cell lines are thought to represent relatively immature neural cells. Cell lines that have a more differentiated character, such as PC12 as well as most glia, do not show regulation by retinoids. We have isolated a series of genes that encode proteins closely related to beta-10. A comparison of their structures reveal a number of interesting features. First, the 3'-flanking regions of the cDNAs are highly conserved (95%) whereas the 5' flanking sequence is not. Second, the 3' region of beta-10 contains a nuclease recognition motif that is absent in thymosin beta-4, a closely related homologue of beta-10. Since beta-4 is not regulated by retinoids neither does its expression change during neurodevelopment, retinoids might act by stabilizing the beta-10 mRNA. The promoter regions of the homologous genes are dissimilar and we are presently determining whether they contain retinoid response elements. In a general sense, the results reported here suggest that retinoids may regulate gene expression, and thereby development, in the early central nervous system.

405.8

PRENATAL COCAINE EXPOSURE INCREASES BRAIN GANGLIOSIDE AND NEUTRAL GLYCOLIPID CONTENT OF NEONATAL RATS. K.C. Leskawa*[1], G.H. Jackson*[1], C.A. Moody*[2] and L.P. Spear[2] (SPON: K. Reid). [1]Dept. Anat. Sci. & Neurobiol., Univ. Louisville Sch. of Med., Louisville, KY 40292, and [2]Dept. Psychol. and Centers for Develop. Psychobiol. and Neurobehav. Sci., SUNY-Binghamton, Binghamton, NY 13901.

Recent studies have reported behavioral dysfunctions in offspring exposed in utero to cocaine, along with alterations in the DA system, but few other biochemical studies have been conducted. Given their role in fiber extension and neuronal maturation, gangliosides and neutral glycolipids were analyzed in whole brains of offspring exposed gestationally to cocaine. Pregnant Sprague-Dawley rats were injected either with 40 mg/kg/3cc cocaine or saline on gestational days 8-20. Offspring were fostered to surrogate dams, sacrificed on postnatal days 1 (P1) and 11 (P11), and brain gangliosides and neutral glycolipids were purified and quantitated. Cocaine-exposed offspring exhibited markedly elevated levels of both total gangliosides and neutral glycolipids at P1 ($p<0.0001$), but not at P11. These transient elevations are in contrast to those reported after fetal alcohol exposure, where decreases in brain gangliosides have been observed. These data suggest that biochemical consequences of gestational exposure to cocaine, or its Ca^{++} chelating metabolite benzoylecgonine, may be far-reaching and not restricted merely to the DA system. Supported by DA04478 (L.P.S.) and NS21057 (K.C.L).

405.9

ANTIBODY 3D10 IMMUNOREACTIVITY IN MONOAMINERGIC NEURONS IN POSTNATAL RATS. S.A. Tobet, J.E. Crandall, R. Whorf*, and T.O. Fox. E.K. Shriver Center, Depts. of Biochemistry and Developmental Neurobiology, Waltham, MA 02254 and *Washington Univ., Div. Biology & Biomedical Sciences, St. Louis, MO 63110.

The 3D10 monoclonal antibody (Tobet & Fox, Dev. Brain Res., In press, 1989) recognizes an antigen(s) in developing monoaminergic cell groups. Significantly more 3D10 immunopositive cells are observed in perinatal compared to adult rats. The experiments reported here examine the neurotransmitter specificity of 3D10 immunopositive cells, as well as the presence of 3D10 immunoreactive antigen(s) in neuronal terminal regions and the protein(s) recognized.

Double-label immunofluorescence reveals that 3D10 is colocalized in multiple catecholaminergic cell bodies in the brainstem, specifically in cells immunopositive against tyrosine hydroxylase (TH) in the locus coeruleus, the sub coeruleus, and the A4 and A5 cell groups. In these regions all 3D10 immunopositive neurons were also TH immunopositive. The reverse was not true, i.e., some TH immunopositive neurons were not 3D10 immunopositive. Further characterization of co-localization with indoleamine cell groups is now in progress. The presence of 3D10 immunopositive neuronal terminals was examined in the cerebral cortex, a major target for fibers of brainstem monoaminergic neurons. 3D10 immunoreactive fibers appeared in different cortical lamina compared to serotonin or TH. Following gel electrophoresis and immunoblot procedures antibody 3D10 appears to recognize a single high molecular weight protein in brainstem regions.

We conclude that monoclonal antibody 3D10 recognizes a protein that is synthesized in developing monoaminergic cell bodies and transported to selected terminal regions. We hypothesize that the developmental disappearance of 3D10 immunoreactivity relates to maturational events in specific monoaminergic circuits. Supported by grants HD20327-TOF/SAT, NS24386-JEC and HD04147-MR.

405.10

EFFECTS OF PROMETHAZINE HCl ADMINISTRATION TO GRAVID RATS ON BRAIN AMINE LEVELS IN OFFSPRING. M.S. Stanford* and J.H. Patton. Department of Psychology, Baylor University, Waco, TX 76798.

Promethazine HCL (Phenergan®, Wyeth) is a phenothiazine widely used for its anti-emetic and sedative potential during human pregnancy. Previous research has suggested that neuroleptic administration to gravid rats results in neurochemical changes in their offspring (Rosengarten & Freidhoff, Science, 203:1133,1978). Sprague-Dawley rats were exposed to Promethazine HCL during both the gestation and lactation stages of development. The drug was injected into dams subcutaneously at a dose of 3.0 mg/kg per day. Offspring's brains were assayed at both 1 and 21 days of age for norepinephrine and dopamine. Assays for serotonin were done on 21 day old brains. Pup brain weights did not differ across groups at either 1 or 21 days of age. Catecholamine assays on 1 and 21 day old brains showed no statistically reliable difference in either NE or DA levels [1 day old brains: NE (Promethazine = 0.0391 µg/gm, Control = 0.0327 µg/gm, p = 0.2010), DA (Pro = 0.0216 µg/gm, Con = 0.0223 µg/gm, p = 0.9355); 21 day old brains: NE (Pro = 0.0873 µg/gm, Con = 0.0958 µg/gm, p = 0.9277), DA (Pro = 0.0348 µg/gm, Con = 0.0383 µg/gm, p = 0.9277)]. Serotonin assays on 21 day old brains did show a statistically reliable difference in serotonin levels (Pro = 0.4212 µg/gm, Con = 0.4936 µg/gm, p = 0.0480) with the promethazine group having 0.0724 µg/gm less brain 5-HT than controls. The results of this study suggest that the 5-HT2 receptor decreasing properties of promethazine HCl affect not only adults (Andree et al., Psychopharm. Bull., 20: 349, 1984) but can affect offspring when exposed transplacentally or through milk.

405.11

COCAINE AND AMPHETAMINE MODULATION OF ULTRASOUNDS AND ACTIVITY IN NEONATAL RATS: DIFFERENTIAL EFFECTS WITH DA AND NE DEPLETION. P.Kehoe,K.Wilson-Miller*,& A.Purbeck*
TRINITY COLLEGE, HARTFORD CT, 06106

Separation from the dam and siblings causes neonatal rats to emit ultrasonic vocalizations. Neurochemically, ultrasounds are influenced by opioids in that morphine decreases and naltrexone increases calling. Endogenous opioids may reduce vocalizations over time. The NE agonist, clonidine, greatly increases calling and NE antagonist, yohimbine, reverses the response and decreases them. Cocaine while increasing activity quiets the isolate. Amphetamine heightens activity and produces a dose-dependent flattening of the normal linear reduction in calling over time. At first the pup calls less but this calling continues over time with no abatement as seen in controls. To differentiate the DA and NE influence on these behaviors, pups were injected ICV on Day 3 with 6-OHDA and preinjected IP with either imipramine (NE uptake inhibitor) or GBR (DA uptake inhibitor). On Day 10 pups demonstrated a dissociation of cocaine's hyperactivity effect in that DA depleted pups were much less active than NE depleted pups who were as active as controls. Ultrasounds after the various treatments presented a more complex profile. In general, pups depleted specifically of NE vocalized less than DA-depleted pups while both groups called less than controls. Cocaine's quieting effects were not seen in DA-depleted infants. The data suggest that isolation calling in rat pups require the presence of NE while cocaine's activity and quieting effects are more specifically DA mediated.

405.12

DEVELOPMENTAL ANALYSIS OF ADENYLATE CYCLASE ACTIVITY AND ITS MODULATION BY SEROTONIN IN THE CNS OF *APLYSIA*. T.N. Chang*, E.A. Marcus, Y. Dudai, and T.J.Carew (SPON: N. Donegan). Depts. Biol. & Psychol., Yale University, New Haven, CT 06520

Sensitization in *Aplysia* is mediated at least in part by serotonin (5HT), which in turn is known to elevate cAMP by activation of adenylate cyclase. Since sensitization does not emerge until relatively late in juvenile development (Late Stage 12, Rankin & Carew, 1988), we were interested in whether development of cyclase activity or its capacity for modulation by 5HT plays a role in the developmental emergence of sensitization. As a first step in this analysis, we have begun to examine cylase activity and its modulation by 5HT at different stages of juvenile development.

Cyclase activity was assessed in whole-CNS homogenates by examining the conversion of [α-^{32}P]ATP into cAMP. To establish normative data, we first examined adult animals. Confirming previous results, significant cyclase activity was detected ($\bar{x}$ specific activity = 40 ± 5 pmol/min-mg protein, $p<.001$, N=12 [6 homogenates]), and was significantly increased by 5HT (10^{-4}M) ($\bar{x}$ increase over basal stimulation = 66%, $p<.003$, N=7 [6 homogenates]). We next examined Early Stage 12 juveniles. Here also, there was significant basal activity of the cyclase ($\bar{x}$ specific activity = 54 ± 4 pmol/min-mg protein, $p<.001$, N=5 [2 homogenates]), which was also enhanced by 5HT ($\bar{x}$ increase = 45%, $p<.02$, N=4 [2 homogenates]). Preliminary experiments show comparable results in Late Stage 12 juveniles.

Our results show that in Early Stage 12 adenylate cyclase is present in the CNS of *Aplysia* and can be modulated by 5HT. Thus far we have only studied the effect of 5HT on whole-CNS homogenates. We are currently examining: (1) other transmitters (e.g. SCP_B), and (2) homogeneous populations of neurons (e.g. sensory neuron clusters), to more quantitatively assess the role of adenylate cyclase and its modulation in the development of sensitization.

405.13

WHAT IS THE RELATIONSHIP BETWEEN THE DEVELOPMENT OF CHOLINERGIC SPINAL NEURONS IN MURINE SLICE CULTURES AND TOTAL CELL PROTEIN PRODUCTION? M.S. Pidherney and H.L. Stewart*, Dept. of Biology, Texas Woman's University, Denton Texas 76204.

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

Cultures were stained for identification of cholinergic neurons at day 0 (day of culturing), three weeks, and five weeks in culture. At corresponding intervals, cultures were incubated in medium containing ^{35}S-methionine and examined for 1) precipitable counts via scintillation counting, and 2) electrophoresis protein patterns. Preliminary data suggest that changes in size and density of cholinergic neurons at the sampled intervals in culture is clearly reflected in total cell protein profile. **(NIH1R29 NS 25250-01)**

405.14

NEONATAL NOREPINEPHRINE (NE) DEPLETION AND ENRICHED HOUSING: HANDLING METHOD IS IMPORTANT! M.J. Saari, B. Buchwald*, K. Fisher*, and J. Kleim*(SPON: B. Pappas).Neuroscience Research Unit, Nipissing University, North Bay, Ontario. P1B 8L7.

This experiment evaluated the effect of two handling methods on the development of enrichment mediated behaviour in NE depleted and vehicle (VEH) injected rats. Newborn male Wistar rats were injected sc., either with 6-OHDA (50 mg/kg) or vehicle (1.0 mg/ml; ascorbic acid in saline) within the first 12 and the first 24 hours after birth. After weaning at day 25, all rats were housed under "enriched" conditions for 35 days (social housing and daily, exposures to an open field containing "toys"). During the daily transfer of the rats into the open field half were lifted by the base of the tail and the rest were lifted while supporting the abdomen. Once a week, the rats were videotaped and the videotapes scored "blind" for rearing, activity and boli. Since the body-lifted rats reared more than tail-lifted the results suggest that different handling methods may influence the development of enrichment mediated behaviour.

405.15

PROTEIN EXPRESSION IN POSTNATAL HIPPOCAMPUS AS VISUALIZED WITH SDS/PAGE. M. Murtaugh*, L.L. Dix*, J.M. Miller*, R.S. Nowakowski+ (Spon: M.F. MacDonnell). *Dept. of Biological Sciences, Rutgers University, Piscataway, NJ 08855, +Dept. of Anatomy, UMDNJ-Robert Wood Johnson Medical School, Piscataway, NJ 08854

Analysis of whole hippocampal tissue from C57Bl/6J or BALB/cByJ mice by polyacrylamide gel electrophoresis (SDS/PAGE) was performed on postnatal days: 0, 2, 5, 14, 38. Using 12% one-dimensional gels, the banding patterns characteristic of the younger ages (P0, P2, P5) were distinct from the banding patterns of the older mice. In the younger samples (P0, P2, P5), six distinct bands were identified which were clearly diminished or undetectable in the older tissue. The bands had approximate molecular weights of: 1) 200-225 kD, 2) 97-100 kD, 3) 62-68 kD, 4) 52-57 kD, 5) 45-48 kD and 6) 28-31 kD. In addition, two bands (approx. 52-57 kD and 45-48 kD) which were absent prior to P5 appeared or became increasingly more visible in the older mice. The differences between the two age groups were clear using silver stained gels but some of the differences could also be detected with Coomassie blue staining. The developmental significance of these differences in banding patterns is as yet unclear. They could represent changes in gene expression or changes in posttranslational modifications of proteins. Further fractionation of these complex protein samples followed by two-dimensional gel electrophoresis may more definitively demonstrate specific changes in protein composition during hippocampal development.

405.16

BRAIN NEUROTRANSMITTERS INCREASE DURING OLFACTORY IMPRINTING IN COHO SALMON *(ONCORHYNCHUS KISUTCH)* AND ARE AFFECTED BY EXPOSURE TO PROPYLTHIOURACIL. S. O. E. Ebbesson, J. Smith, C. Co*, and L. M. Cheek*. Institute of Marine Science, University of Alaska Fairbanks, Fairbanks Alaska 99775,USA and Department of Psychiatry, LSU School of Medicine, Shreveport, LA 71130, USA.

About the time coho salmon head for the ocean some 18 months after hatching a smolt transformation (ST) occurs that include a color change, the development of salinity tolerance, downstream migratory behavior, and conversion from territorial behavior to schooling. High plasma thyroxine levels at this time suggests the involvement of this hormone in some aspects of ST, including the olfactory imprinting. We report here that whole brain content (as determined by HPLC) of dopamine (DA), norepinephrine (NE), serotonin (5-HT), 5-HIAA, and glycine (Gln) increase significantly during this period of neural plasticity while other putative transmitters such as glutamate, aspartate and GABA show no such change. The ratio of 5HT/5HIAA as an indicator of serotonin turnover is also increased during the ST. The data suggest that 5-HT, DA, NE and Gln may be involved in the neural mechanisms underlying olfactory imprinting, behavioral changes and neural plasticity associated with smolt transformation. We also report here that plasma thyroxine levels increase dramatically at the beginning of ST, about a week before whole brain content of serotonin (5-HT), dopamine (DA), norepinephrine (NE) and glycine (Gln) increase suddenly and decrease to normal levels in 14 days. Fish treated with 10 ppm propylthiouracil showed essentially no increase in plasma T_4 levels, yet the brain levels of 5-HT, DA, NE and Gln spiked at the same time and to the same extent as the controls. Return to normal brain levels was significantly delayed in PTU treated fish demonstrating that this substance has very specific effects on the brain.

Supported by grants from NIH and Alaska Sea Grant College Program.

405.17

CHRONIC PERINATAL NALTREXONE ADMINISTRATION DECREASES NEURONAL PACKING DENSITY. J.V. Seatriz* and R.P. Hammer, Jr. Dept. Anatomy & Reprod. Biol., Univ. Hawaii Sch. of Med., Honolulu, HI 96822.

It has been suggested that endogenous opioid peptides may act as inhibitory trophic factors in the central nervous system during ontogeny. This study examined the effect of chronic opiate receptor blockade on packing density of neurons and glial cells in various brain regions.

Osmotic minipumps (Alzet, 2ML4) which administered 10 mg/kg/day naltrexone or saline vehicle until weaning were implanted subcutaneously on gestation day 12 in timed-pregnant Sprague-Dawley rats. Litters were culled to 8 at birth. Male pups were anesthetized by hypothermia at postnatal day 6 and perfused intracardially with 10% neutral buffered formalin, brains were removed to postfix and graded sucrose solutions, blocked and sectioned at 20 m on a freezing microtome, and sections were mounted and stained with thionin. Neuronal and glial packing density was determined by cell counts using calibrated, high resolution microscopy in each layer of primary somatosensory (S_1) and motor (M_1) cortices and in the preoptic area of the hypothalamus (POA). Constant perinatal opiate receptor blockade significantly reduced neuronal packing density in medial (42%) and lateral (36%) POA, layers II-V of S_1 (18-35%), and layer V of M_1 (33%). Glial cell packing density, however, was unaffected by naltrexone treatment in any region.

Thus, naltrexone treatment during the cell proliferation and growth period has effects which are selective by cell type and brain region. It is unclear, as yet, whether reduced neuronal packing density reflects increased cortical thickness or reduced neuronal number. (Supported by USPHS awards DA04081, NS01161, RR08125 and RR03061.)

405.18

CREATINE KINASE ACTIVITY IN PRIMARY CULTURES OF NEURONS, ASTROCYTES AND OLIGODENDROCYTES. P. Manos* and J. Edmond* (SPON: G. K. Bryan) Dept. of Biol. Chem., UCLA School of Medicine, Los Angeles, CA 90024

Creatine kinase (CK) catalyzes the reversible transfer of a high energy phosphate from ATP to creatine. The BB-isozyme is present in high quantities in brain and is thought to play a crucial role in energy maintenance reactions. Primary cultures of purified populations of neurons and glia, derived from embryonic and neonatal rat cerebra, respectively, were used to elucidate the cellular compartment and function of CK in developing brain. Cytosolic CK activity in neurons reached maximal levels of 4 U/mg protein by Day 4, and stayed constant throughout the 14 day culture time. The CK activity in astrocytes cultured for 20 days was comparable to the activity determined in whole brain at the time of birth (3 to 4 U/mg), and was 1.5-fold higher than the activity determined at the time the cells were isolated. In contrast, the CK activity in oligodendrocytes (Day 20) was about double that found in the mixed glia cultures, 3- to 4-fold higher than that determined in astrocytes and almost 7-fold higher than the activity measured when the cells were isolated. The activity of the myelin marker 2',3'-cyclic nucleotide phosphohydrolase was over 10-times greater in the oligodendrocytes than in astrocytes. The relatively higher levels of CK in oligodendrocytes indicate that CK may have an additional role in myelinogenesis. (Supported by NIH grant HD 06576).

DIFFERENTIATION, MORPHOGENESIS AND DEVELOPMENT: CHANNELS AND CURRENTS

406.1

DEPOLARIZATION INDUCED CHANGES IN SYNAPTIC VESICLE PROTEIN (P65) EXPRESSION IN SCG EXPLANTS. K.M. Linderman* and K.F. Greif. Bryn Mawr College, Bryn Mawr, PA 19010.

P65 is an integral membrane protein of synaptic vesicles which appears in synaptic vesicles of various transmitter types. We have shown p65 to be regulated transsynaptically in the superior cervical ganglion (SCG) of adult and neonatal rats (Greif and Trenchard, Synapse 2:1, 1988). Pharmacological studies suggest that impulse activity is at least partially responsible for this transsynaptic regulation in vivo (Greif, J. Neurosci. 6:3628, 1986).

In vitro studies in defined medium support the role of depolarization in the regulation of p65 levels. Neonatal SCG explants treated with the sodium channel ionophore, veratridine, or elevated potassium express 60% more p65 than controls. This effect is reversible by tetrodotoxin treatment.

P65 was metabolically labelled in neonatal SCG explants to determine the role of new protein synthesis in the increases of p65 caused by depolarization. SCG explants were treated with veratridine for 36 hours and then pulse-labelled with ^{3}H-leucine for two hours in the presence or absence of cycloheximide, a protein synthesis inhibitor. P65 was purified on a monoclonal antibody affinity column. Elutions were counted for two minutes on a scintillation counter. Veratridine treatment significantly increases ^{3}H-p65 over control levels by 64% (SEM=7, $P<0.005$, t-test). Concurrent cycloheximide treatment with veratridine lowers ^{3}H-p65 levels by 43-105%. Results suggest that increases of P65 seen after depolarization treatments are due to an increased rate of synthesis and not to the unmasking of a preexisting pool of antigen.

Supported by Dysautonomia Foundation, Inc. and NSF grant BNS 85-19673 to KFG.

406.2

DIFFERENTIATION AND SURVIVAL OF ACETYLCHOLINESTERASE-POSITIVE NEURONS IN CULTURE: THE ROLE OF CHEMICAL DEPOLARIZATION AND COCULTURE WITH ASTROCYTES J.Dymshitz*, R.Malach, S.Amir and R. Simantov (SPON:S.Yehuda) Dept. of Neurobiology and Genetics, Weizmann Inst. Rehovot, Israel

Chemical depolarization of rat striatal primary cultures was used as a model for studying the influence of neuronal activity on the survival and differentiation of acetylcholinesterase (AChE)-expressing neurons. Embryonic (E15) striatal cells were plated on poly-ornithine or on a monolayer of astrocytes and cultured in serum-free or serum-containing medium. In all the four combinations veratridine had a dose-dependent inhibitory effect on AChE-expressing cells; at 5μM there was a loss in AChE-containing neurites whereas a higher concentration of veratridine led also to a marked reduction in the number of stained cell bodies. These effects could be elicited also by 40-60 mM KCl. The neurite loss induced by 5μM of veratridine was reversible, so that a partial recovery has been observed after veratridine removal. Blockers of voltage-sensitive Ca^{++} channels - verapamil and nifedipine - did not alter the effect of veratridine, while TTX blocked partially veratridine effect. These observations and the finding that astrocytes increased the basal number of AChE-positive cells in serum-free cultures indicate that the neuronal activation state and the interaction with glial cells affect the survival and expression of AChE-positive neurons.

406.3

CYCLIC AMP MIMICS NERVE GROWTH FACTOR IN THE INDUCTION OF SODIUM CHANNELS IN PC12 CELLS. D. Kalman[1], B. Wong[1], M. J. Cline[2], and P. H. O'Lague[1*], Departments of Biology[1] and Hematology-Oncology[2], University of California at Los Angeles, Los Angeles, CA 90024.

Nerve growth factor (NGF), a trophic agent for several classes of vertebrate neurons, acts as a differentiation signal for PC12 cells, a model neuronal system. NGF causes neurite outgrowth and expression of voltage-gated Na^+ channels. Neurite outgrowth involves in part activation of protein kinase C (PKC), but mechanisms controlling channel expression are not known. Using patch-clamp techniques to assay for functional channels, we found that agents activating cAMP-dependent protein kinase (PKA), but not PKC, mimicked NGF's ability to induce channels. p21, a ras oncogene product that can activate PKC and mimic NGF-dependent neurite outgrowth, also did not induce channels. Thus, Na^+ channel induction and neurite outgrowth in PC12 cells appear to occur by activation of different protein kinases. Supported by NSF (88-20861), NCI (CA15619) and the Giannini Foundation.

406.4

IN VIVO DEVELOPMENT OF POTASSIUM CURRENTS IN EMBRYONIC CHICK LIMB MOTONEURONS. D.P. McCobb and K.G. Beam. Physiology Dept., Colorado State Univ., Fort Collins, CO 80523.

The differentiation of many aspects of both muscle and motoneuron phenotype in the chick have been shown to be controlled by functional activity of the neuromuscular system. We have previously described developmental changes in Ca++ and Na+ currents in chick limb motoneurons which take place while other aspects of the system are developing, and might therefore control (and/or be controlled by) these other developmental events. Here we report large changes in motoneuronal K+ current expression, an immediate consequence of which is a maturational change in the action potential waveform. Whole-cell patch clamp methods were used to study voltage dependent K+ currents and action potentials in limb motoneurons isolated from E4, E6, and E11 chick embryos (HH stages 21-23, 28-29, and 37, respectively). During this span limbs differentiate from primordia to well-defined and motile structures. All recordings were made on the day following dissociation. Limb motoneurons were identified by retrograde labelling with the carbocyanine dye, DiI. Delayed rectifier type K+ currents (I_K), which were greatly reduced by TEA+, were relatively large even at E4, and increased moderately (25%) by E11. By contrast, A-type K+ current (I_A, identified by rapid voltage dependent inactivation and 4-AP sensitivity) was small or absent in E4 motoneurons, and increased greatly (~16 fold) by E11. The action potential duration was greatly affected by 4-AP but not TEA+, and was found to decrease during development by approximately 50%. We conclude that the development of I_A in embryonic motoneurons reduces the duration of the action potential, which, by affecting synaptic transmission, might influence activity-dependent developmental events in nerve or muscle. We are currently studying the effects of blocking neuromuscular transmission on the development of K+ and other currents in motoneurons using curare-induced paralysis.

This study was supported by NIH grants NS 07383 to DPM and NS 24444 to KGB.

406.5

MEMBRANE POTENTIAL RESPONSES OF EMBRYONIC RAT SPINAL CORD CELLS ANALYZED WITH DIGITAL IMAGING MICROSCOPY. M.K. Walton, A.E. Schaffner, and J.L. Barker. Laboratory of Neurophysiology, NINDS, NIH, Bethesda, MD 20892.

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

Rat spinal cords from embryonic days 13, 15 and 17 (E13, E15, E17) were enzymatically dissociated into single cell suspensions, placed in culture dishes and allowed to adhere for ca. 2 hours. Culture dishes were perfused with continuously flowing solutions containing 100 nM of the voltage sensitive oxonol dye $DiBaC_4(5)$. These cells all showed reproducible and reversible changes in fluorescence indicating membrane depolarization in response to increases in extracellular potassium concentration. Veratridine was used as a probe for the voltage sensitive sodium channel. The depolarizing response to veratridine was largely absent from cells dissociated at E13 but developed progressively so that by E17 almost all cells responded. This response was blocked by tetrodotoxin or use of sodium-free medium.

This preliminary data suggests that the sodium-selective voltage-sensitive channel activated by veratridine develops subsequent to E13 in the rat spinal cord, in agreement with previous reports using flow cytometry (Mandler, et.al., (1986) Neurosci. Abs. 12:1349). This technique will be useful for investigations of cultured cells as it allows for simultaneously recording multiple cells while at the same time monitoring each individual cell's response to successively applied conditions.

406.6

DEVELOPMENT OF VOLTAGE-GATED ION CHANNELS IN DIFFERENTIATING HYPOTHALAMIC NEURONS. **R. E. Petroski and H. M. Geller. Department of Pharmacology, UMDNJ Robert Wood Johnson Med. Sch. and The Graduate School, Rutgers University, Piscataway, NJ 08854.**

Neuronal proliferation occurs between E13 and E17 in the rat hypothalamus. Following terminal division, neuronal precursors undergo the complex process of cytodifferentiation producing a heterogeneous population of neurons. This process involves the expression of gene products which confer the mature neuronal phenotype. In order to study the events of neuronal differentiation, we use the embryonic rat hypothalamus as a source of precursors and newly differentiating neurons. We employ the whole cell patch-clamp technique to examine ion channel expression during the first week of development *in vitro*.

Recordings could be obtained from neurons as early as five hours following dissociation. These cells are small, round (10μm dia) and have one or two processes which are starting to "bud." In the first 24 hours, none of the neurons expressed Na^+ currents; 50% of these neurons exhibited small (50 pA) outward currents carried by K^+. At 1-3 d.i.v., neuronal somata grow to a maximum 15-20 μm dia. and acquire a more mature neuronal morphology: bipolar, tripolar and pyramidal shapes with longer processes. At this time Na^+ currents could be detected in 50% of the neurons and all neurons express sustained outward currents; additionally, an inactivating outward component is seen in most cells. The inactivating component is quite large relative to the sustained component when measured immediately after rupturing the cell membrane. However it "runs down" quickly suggesting that it may be Ca^{2+}-dependent. By the 4th day in culture, all neurons display Na^+ currents. Over this time course, ion channel density increases since the amplitude of both inward and outward increases until a steady state is achieved at about one week in culture.

These data taken together with our immunocytochemical studies support a coordinated temporal expression of ion channels and neurotransmitter phenotype in developing hypothalamic neurons in culture. Supported by NIH.

406.7

ANALYSIS OF CALCIUM FLUXES IN DISSOCIATED RAT NEURONS DURING EMBRYONIC DEVELOPMENT. **S. M. O'Connell*, J. P. Grierson, R. E. Petroski and H. M. Geller. (SPON. G. S. F. Ling) Department of Pharmacology, U.M.D.N.J. - Robert Wood Johnson Medical School and The Graduate School, Rutgers University, 675 Hoes Lane, Piscataway, NJ 08854.**

The development of functional voltage-sensitive Ca^{++} channels (VSCC) in the embryonic rat brain can be investigated using the technique of flow cytometry and the fluorescent probe, INDO-1. This approach obviates the possibility of premature differentiation when embryonic cells are placed into tissue culture. Freshly isolated cells were incubated in INDO-1,AM for 30 minutes followed by a rinse and a 30 minute post-incubation period. The efficiency of INDO-1 hydrolysis was tested for each experiment by permeabilizing to calcium with ionomycin. Analysis of dissociated cell preparations from the E14-E17 hypothalamus reveals that about 25 to 30% of cells respond to a stimulus of 25 or 50 mM KCl with a significant influx of Ca^{++}, whereas only 10 to 15% of cells obtained from the cerebral cortex are responsive under the same conditions and from the same embryonic ages. The Ca^{++} flux in dissociated cells from both regions is markedly attenuated by 1 μM nifedipine, however only the cortical cells appear to be potentiated by 1 μM Bay K. The responding cells from both regions have been shown to be neurons by sorting on the basis of $[Ca^{++}]_i$ maintaining in culture and then staining for MAP2 immunoreactivity. Furthermore, the detection of K^+-stimulated Ca^{++} uptake by astrocytes is believed to be negligible, since experiments with cultured astrocytes indicate a very low level of INDO-1 hydrolysis by these cells. It can be inferred that cells from the embryonic brain preparations also express voltage-sensitive sodium channels (VSSC) since the addition of tetrodotoxin attenuates the Ca^{++} response when compared with KCl alone, suggesting that in the control situation depolarization is augmented by Na^+ influx through VSSC. Supported by NIH NS25168.

406.8

DEVELOPMENT OF SPONTANEOUS ACTIVITY OF HYPOTHALAMIC NEURONS IN DISSOCIATED CULTURE. D.S.F.Ling, R.E.Petroski, W.Chou*, H.M.Geller. Department of Pharmacology, UMDNJ-Robert Wood Medical School and Graduate Programs in Biomedical Engineering and Pharmacology, Rutgers University, Piscataway, N.J. 08854.

Past studies have shown that many neurons in the hypothalamus exhibit spontaneous, phasic discharge of action potentials. Such neurons have been been associated with temperature control and regulation of hormone release. We have exmained the development of sponataneous firing in dissociated hypothalamic neurons using a loose patch recording method. Hypothalami were dissected from E17 rat brains and plated onto a confluent monolayer of cortical astrocytes. Extracellular recordings were made from neurons, which are 10-25 μM diameter, with patch electrodes beginning 1 day *in vitro*. Seal resistances ranged from 20-80 MΩ on these neurons in this recording mode, with optimal input resistances ranging from 40-60 MΩ. Over the course of 6 weeks *in vitro*, 45 of 165 neurons exhibited spontaneous discharge of action potentials. The percentage of neurons displaying spontaneous discharge patterns increased with time in culture, suggesting a developmental aspect to the production of action potentials. There did not appear be any correlation between basal firing frequency and time in culture. Preliminary studies using high Mg^{2+}/low Ca^{2+} bath solutions suggest that spontaneous activity in young neurons may not be dependent on synaptic activity.

The results of this study taken together with our patch clamp and immunocytochemical studies suggest that spontaneous activity in culture develops after ion channel expression and neurotransmitter synthetic activity. (Supported by NIH NS 25168)

NEUROTOXICITY: METALS AND ORGANICS

407.1

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

Hydrogen sulfide (H_2S) is an environmental pollutant which can produce severe effects on the adult CNS;however, little is known as to its effects on the developing CNS. Pregnant rats (Sprague-Dawley) were exposed to either 20 pmm or 50 pmm H_2S for 7 hours per day in an environment chamber from Day 7 postcoitus until Day 21 postnatal. Controls were similarly treated including placement in an environmental chamber flushed with room air. On Day 21 postnatal, representative pups from each litter were euthanized, the cerebella removed and processed for Golgi staining. Ten complete Purkinje cells were selected from each group and analysed, using vertex analysis (Berry & Finn, Proc. R. Soc. Lond. B. 221:321, 1984). At both exposure concentrations, there was a significant increase in vertex path length suggesting an increased distance between each new generation of branches. New growth occurred toward the pial surface and internally but was restricted laterally. The treated cells exhibited an unusually high non-random growth pattern. In summary, both treatments produced significant growth changes in the dendritic arborization. (Supported by Alberta Occupational Health and Safety Heritage Grant Program).

407.2

POSTNATAL LEAD EXPOSURE OF RATS DOES NOT PERTURB HIPPOCAMPAL ZINC CONCENTRATION. E. J. Kasarskis and T. M. Forrester*. Dept. Neurology and Toxicology, VA and Univ. of Kentucky Med. Ctrs., Lexington KY 40536-0084.

Postnatal lead (Pb) exposure alters the development of the hippocampus, a region of the brain rich in zinc (Zn). Because Pb is sequestered in this region, others have proposed that the manifestations of Pb toxicity may be caused by a Pb-induced reduction of hippocampal Zn. To test this hypothesis, we have measured Pb and Zn in microdissected hippocampal subfields after postnatal Pb.

Lactating dams ingested water containing either 0.2% or 2.5% Pb after parturition. Controls were either offered deionized water and food ad libitum or were pair-fed to the 2.5% Pb group. Pups were sacrificed at weaning. Hippocampal subfields were microdissected and analyzed for Pb and Zn using atomic absorption spectrophotometry. Despite a 6.3 fold accumulation of Pb in the 2.5% Pb group compared to controls, the concentration of Zn in the whole hippocampus was not perturbed. Moreover, the concentration of Zn in any hippocampal subfield was not altered by Pb exposure during postnatal development. Although Zn and Pb are both enriched in the hippocampus, these data indicate that they probably do not compete for binding to the same ligands. These results compliment the study of Petit and LeBoutillier (Neurotox 7:237, 1986) which demonstrated behavioral dissimilarities between postnatal Zn deficiency and Pb toxicity.

407.3

NEUROCHEMICAL EFFECTS OF PRENATAL LEAD EXPOSURE IN GUINEA PIGS. T.K. Rowles*, W.D. Blaker, and E. Tiffany-Castiglioni*. Va-Md Regional College of Veterinary Medicine, Blacksburg, Va 24061.

Blood lead (Pb) levels correlate with cognitive dysfunction in children, and umbilical cord blood levels indicate many children are exposed *in utero* to significant Pb levels. Since the effects of such exposure are still unclear, we are using the guinea pig as an animal model of prenatal exposure to further study this problem.

Guinea pigs were exposed to one of 3 levels of Pb daily during the last 2 trimesters of pregnancy. On postnatal day 38, their offspring were killed by head-focussed microwave irradiation and specific brain regions collected for analysis of neurotransmitter levels by HPLC-ED. Lead exposure resulted in no changes in maternal weight gain, litter size, birth weight, or neonatal growth rates. However, lead exposure did result in significant increases in metabolite levels of dopamine and serotonin in several brain regions. HVA levels increased with Pb treatment in frontal cortex, striatum, septum, thalamus, and inferior colliculus, but DOPAC levels did not change. Dopamine levels decreased only in the inferior colliculus. 5-HIAA levels also showed widespread increases, but serotonin levels did not change. There appears to be an increased metabolism of these neurotransmitters in animals exposed prenatally to Pb. Funded by BRSG, Virginia Tech.

407.4

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

A consistent feature of the aluminum-induced encephalomyelopathy of rabbits is the presence of neurofibrillary inclusions in motor neurons. To investigate the mechanisms underlying this phenomenon, we exposed fetal rabbit motor neuron-enriched cultures to varying concentrations of aluminum chloride.

At 16 days in culture, when all major neurofilament subunit proteins are present, the media was supplemented with either 10 mM, 1 mM or 100 uM aluminum chloride. Within 48 hours, the cultures exposed to 10 mM and 1 mM aluminum chloride developed refractile intracytoplasmic inclusions and rare focal neuritic swellings. By 96 hours these features were present in all cultures, with motor neurons exposed to 10 mM aluminum chloride developing signs of cell death. The inclusions demonstrated immunoreactivity with monoclonal antibodies against phosphorylated neurofilament.

These observations demonstrate that dissociated fetal rabbit motor neurons *in vitro* recapitulate the aluminum-induced neurofibrillary inclusions of rabbit motor neurons *in vivo*, thereby providing a dynamic model of aberrant neurofibrillary degeneration in nontransformed cells. (*Supported in part by a research fellowship from the Medical Research Council of Canada)

407.5

DEPRESSED HIPPOCAMPAL AND BASAL FOREBRAIN ACTIVITY OF CHOLINE ACETYLTRANSFERASE IN 15 DAY OLD PUPS FROM RATS INGESTING POLYCHLORINATED BIPHENYL. L.A. Meserve, L.M. Juarez de Ku, B.B. Surbeck*, C.A. Gaskins*, and J.A. Landis*.
Dept. Biol. Sci., BGSU, Bowling Green, OH 43403-0212

Polychlorinated biphenyl (PCB) is a ubiquitous environmental pollutant, the ingestion of which functions to the detriment of a number of physiological systems in adult animals. Recent studies in our lab have shown PCB feeding to pregnant and lactating rats to depress circulating thyroxine levels in their 15 day old pups. Since hypothyroidism has been shown to depress choline acetyltransferase (ChAT) activity in young rats it was of interest to determine whether PCB, administered through the mother, would have a similar effect. Female rats were fed PCB (Arochlor 1254) as 0.25% of a standard diet through pregnancy and lactation. Fifteen day old pups were decapitated, and the activity of ChAT in hippocampus and basal forebrain was estimated by the ability of an homogenate to incorporate ^{14}C-labelled acetyl-CoA into acetylcholine. When activity was expressed as nm labelled product generated/mg soluble protein/hr, PCB depressed hippocampal ChAT to about 60% of normal and that in basal forebrain to 40%. Thus it appears that PCB has undesirable effects on brain neurotransmitter systems. This may result from concomitant hypothyroidism or direct PCB effects. (Supported by BRSG #89-02).

407.6

POLYCHLORINATED BIPHENYLS DECREASE SPONTANEOUS AND D-AMPHETAMINE INDUCED LOCOMOTOR ACTIVITY IN THE ADULT RAT. R.F. Seegal, K.O. Brosch* and R. Okoniewski*. Wadsworth Center, NY State Dept. Health, Albany, NY 12201-0509.

Exposure of adult rats to polychlorinated biphenyls (PCBs) decreases regional brain biogenic amine function (Seegal et al., NSA, 14, Part 2 p. 885, 1988). To determine the functional significance of these changes we have examined the effects of PCBs and catecholaminergic drugs on locomotor activity.

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

Spontaneous activity in the PCB-exposed animals was significantly lower than in control animals. D-amphetamine significantly increased activity although PCBs attenuated the drug-induced increase in locomotor activity.

Thus, PCB exposure of the adult rat decreases both spontaneous and drug-induced locomotor activity. These effects on locomotor activity may be causally related to concomitant decreases in central catecholamines.

407.7

AGE-RELATED CHANGES IN CAPSAICIN-INDUCED DEGENERATION IN RAT BRAIN. T. Dinh* and S. Ritter (SPON: M. Hyde). Dept. of VCAPP, Washington State University, Pullman, WA 99164-6520.

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

408.1

THE EFFECT OF CHRONIC ETHANOL CONSUMPTION ON CELL NUMBER IN THE MOUSE CEREBELLAR CORTEX, RMA Napper*and RJ Harvey
Dept Anatomy and Neuroscience Centre, Univ. of Otago, Dunedin, New Zealand.

In the present study, quantitative methods have been used to determine the changes in layer volumes and numbers of cells in the cerebellar cortex of adult mice following long term ethanol consumption. Three month old male BALB/C mice were exposed to ethanol vapour for nine hours per day. This regime was used on two groups (n=32) for 3 weeks; one group (A) was killed at the end of 3 weeks ethanol exposure while the second (B) had a 3 week 'ethanol free' recovery period (Phillips & Cragg, J.Stud.Alc., 45:475, 1984). Two further groups acted as controls. Individual layer volumes, Purkinje cell (Pcell) layer areas, total cerebellar volumes and Pcell numbers were estimated using the methods of Harvey and Napper (J.Comp. Neurol., 274:151, 1988). Cerebellar volumes (mm^3), cell layer areas (mm^2), Pcell densities ($x10^3/mm^2$) and Pcell total numbers ($x10^5$) (n=4) were respectively; 41.9±3.4, 157.2±5.0, 2.1±0.2, 3.24 (mean±s.d) (A-control); 37.9±2.9, 146.3±10.6, 2.2±0.3, 3.23 (A-alcohol); 40.5±8.0, 152.8±8.6, 2.0±0.3, 3.03 (B-control); and 40.0±6.7, 155.2±26.1, 2.1±0.3, 3.27 (B-alcohol). There is no significant difference in cerebellar volumes, Pcell layer areas, or total numbers in either group A or B. These results do not support the conclusions of Phillips & Cragg (op.cit.) and Tavares et.al. (Alc:Clin.Exp.Res., 11:315, 1987).

408.2

A MODEL TO STUDY THE EFFECTS OF CHRONICALLY INFUSED NEUROPHARMACOLOGICAL AGENTS ON DEVELOPING EMBRYOS. R. Roll*, T.A. Jones, UNMC College of Dentistry, Lincoln, NE 68583-0740 and B.J. Morley, Boystown Nat. Inst., Omaha, NE 68131.

The purpose of this investigation was to develop an experimental model that could be used to assess the effects of neuropharmacological agents on developing embryos in vivo. A method of chronic in ovo drug infusion was developed (fertilized eggs, *Gallus domesticus*). Neurophysiological effects of drug administration were assessed by recording compound action potentials of the vestibular nerve (Jones & Pedersen, Am. J. Otolaryn. in press, 1989). Nicotine (NIC) was chosen for the present study. Animals were divided into four groups: 1) control, 2) sham (vehicle only), 3) continuous drug and 4) drug followed by vehicle only. Catheters were implanted in 18 eggs and sealed in place under sterile conditions to enable continuous infusion of drug and vehicle (1cc 6.03 mg/ml over 10 days). The resulting nicotine levels in blood serum (SER) and extra- embryonic fluid (EEF) were determined (SER: 21.4 ± 6.5 ng/ml, COT 10.6 ±1.0 ng/ml; EEF: 97.0 ± 89.5 ng/ml). On the nineteenth day of development, the eggs were opened to access embryos and subcutaneous skull electrodes were placed. Vestibular response thresholds and input/output functions were determined. The animals were then decapitated, brains removed and prepared for receptor binding studies. H_3^* -nicotine was used in binding assays to estimate brain nicotinic acetylcholine receptor densities. The study demonstrates the usefulness of the avian model in research evaluating the consequences of exposing embryos to neuropharmacological agents.

408.3

THE EFFECT OF PRENATAL COCAINE EXPOSURE ON UMBILICAL CORD LENGTH IN FETAL RATS. S. Barron*, J.A. Foss* and E.P. Riley. Psychology Dept. University of New York, Albany, NY 12222.

With the dramatic escalation of cocaine abuse, there is increased concern regarding its' teratogenic potential. It has been suggested that cocaine may produce brief periods of fetal hypoxia. Since fetal hypoxia may be associated with reduced fetal movements, this study examined the effects of prenatal cocaine exposure on an indirect measure of fetal movement, umbilical cord lengths. Pregnant rats were intubated with either 0 or 60 mg/kg cocaine hydrochloride daily from gestation day (GD) 12 - 21 and umbilical cord lengths were measured on GD 21. Fetuses exposed to cocaine in utero had shorter umbilical cords than intubated controls. There were no differences in either placental or fetal body weights across the prenatal treatment groups suggesting that the cord length differences were not simply due to weight differences. These data suggest that maternal cocaine consumption may interfere with normal fetal movement. This work was supported in part by NIDA grant #DA04275 to EPR.

408.4

SWIMMING BEHAVIOR IN MICE SIRED BY ALCOHOL CONSUMING FATHERS. P.J. Bilitzke and E.L. Abel
Dept. of Ob/Gyn, Wayne State University School of Medicine, Detroit, MI 48201.

Male mice consumed liquid alcohol diets containing 25%, 10% or 0% ethanol derived calories (EDC). Animals receiving the 10 and 0% EDC diets were pair fed to those consuming the 25% EDC diet. After seven or fourteen weeks of consumption, males were bred to non-treated females. Offspring were tested for swimming behavior at 75 days of age. Offspring sired by alcohol-consuming males were more immobile regardless of duration of paternal alcohol consumption or housing conditions (group or isolated). Imipramine (5, 15 mg/kg) reversed this effect such that after drug treatment, alcohol-sired offspring were less immobile than controls. Propranolol (1, 3 mg/kg) eliminated the group differences. Yohimbine (1, 10 mg/kg) significantly increased immobility in all groups but did not reverse the effects of paternal alcohol consumption. Metergoline (1 mg/kg) increased immobility in all groups but did not reverse the effects of paternal alcohol exposure. AMPT (100 mg/kg) did not affect swimming behavior. These results extend the evidence for paternally mediated behavioral mutagenesis. Supported by P50 AA07606 and AA 06999.

408.5

EFFECT OF PRENATAL EXPOSURE TO ETHANOL ON GENE EXPRESSION DURING RAT BRAIN DEVELOPMENT. D. Maciejewski-Lenoir* and R. J. Milner.
Research Institute of Scripps Clinic, La Jolla, CA 92037.

There is considerable evidence that prenatal exposure to ethanol results in abnormal development of the nervous system. To investigate this phenomenon at the molecular level, we have analysed the effect of *in utero* exposure to ethanol on the postnatal expression of particular brain genes. Pregnant rats were exposed to ethanol vapor between days 8–21 of gestation, resulting in blood alcohol levels between 150 and 200 mg%. At birth, pups from the ethanol-treated rats and from untreated control animals were fostered to normal mothers. Ethanol-treated and control pups were sacrificed at different times after birth and RNA was extracted from four dissected brain regions: hindbrain, cerebellum, midbrain and cortex. The expression patterns of particular mRNAs were analysed on Northern blots and by solution hybridization. The mRNAs tested were chosen to represent genes expressed in different CNS cell types and included mRNAs encoding MAG/1B236, PLP, Tα1-tubulin and GFAP. The results show differences in mRNA expression between control and ethanol-exposed animals with some variation among brain regions. In general, the data indicate that ethanol causes a slowing or delay in the normal time course of expression of the marker genes during brain development and differences in their adult levels of expression. Supported in part by NIAAA Alcohol Research Center grant AA06420.

408.6

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

The most serious aspects of fetal alchol syndrome (FAS) in humans are long-term deficits in cognitive function. Adequate models of permanent cognitive deficits in animals produced by alcohol exposure during development have been difficult to develop. We report here that alcohol exposure to neonatal rats during the brain growth spurt, comparable to that of the human third trimester, results in severe learning deficits in a radial maze task, including working memory deficits correlated with hippocampal cell loss in CA1. During postnatal days 4-9, rat pups were exposed to 7.5 g/kg (n=14) or 6.6 g/kg (n=16) of alcohol per day using artificial rearing procedures, which resulted in peak blood alcohol concentrations of 415 and 335 mg/dl, respectively. Control groups included artificially reared gastrostomy controls (n=16) and normally reared suckle controls (n=18). As adults, they were tested for 30 days on a 12-arm radial maze, in which six arms were consistently baited and six were unbaited. The alcohol-treated rats committed significantly more errors related to working memory (within-session repeated arm entries), and also more errors related to reference memory (initial entries into unbaited arms). However, the working memory deficits were much more severe. Cell counts on the rats sacrificed at 200 days old indicated that within the hippocampal formation, significant cell loss occurred only for field CA1 pyramidal cells, which were reduced by 16%-20%. There was a significant negative correlation (r=-.578) of working memory errors with CA1 cell counts, suggesting that in this model of FAS, deficient spatial information processing is associated with CA1 hippocampal cell loss. (Supported by NIAAA grants #AA05523 and AA07313).

408.7

ALCOHOL EXPOSURE DURING THE BRAIN GROWTH SPURT INDUCES PERMANENT MICROENCEPHALY AND NEURONAL DEFICITS IN RATS. J.R. West, D.J. Bonthius and C.R. Goodlett. (SPON: J.D. Coulter). Dept. of Anatomy, University of Iowa, Iowa City, IA 52242.

A rat model of alcohol-related developmental effects was used to examine the long-term morphological changes associated with developmental alcohol exposure. Sprague-Dawley rat pups were reared artificially over postnatal days (PD) 4-12 (a period of rapid brain growth similar to the human third trimester). On PD 4-9, alcohol-exposed groups received either 6.6 or 7.5 g/kg/day ethanol (administered as 7.5% and 8.5% v/v solutions, respectively) in 4 of the 12 daily feedings. Controls included artificially reared (gastrostomized) controls and normally reared suckle controls. Pups were fostered back to dams on PD 12, weaned on PD 21 and perfused on PD 200. Brain weights were measured. Cerebellar Purkinje cells in all 10 vermal lobules and hippocampal neurons in fields CA1, CA2/3 and CA4 were counted from single two-micron-thick sections. While adult body weights were not affected by the alcohol treatments, total brain weights were significantly smaller in both alcohol-treated groups, relative to controls. Both alcohol treatments resulted in permanent neuronal deficits. In the hippocampus, CA1 pyramidal cells were significantly reduced by both alcohol treatments, while CA2/3 and CA4 were not reduced by either treatment. There was a significant loss of Purkinje cells in the cerebellum, and some lobules were significantly more affected than others. The lobules in which Purkinje cells were most mature at the time of alcohol exposure (lobules I, II, IX and X) were the most vulnerable to Purkinje cell loss. Therefore, developmental alcohol exposure induces permanent neuronal loss which is population- and region-specific. (Supported by NIAAA grant AA05523.)

408.8

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

The cerebellum in the neonatal rat is highly susceptible to ethanol-induced Purkinje cell death following exposure on postnatal days (PD) 4-10. We examined whether ethanol exposure on a single day during the brain growth spurt could induce Purkinje cell loss. Rat pups were assigned to one of four groups on PD 4: 6.6 g/kg/day ethanol group, 3.3 g/kg/day ethanol group, gastrostomy and suckle control groups. The ethanol and gastrostomy control groups were reared artificially. Ethanol-treated groups were given two consecutive ethanol-containing feedings on PD 4. All subsequent feedings were with milk formula alone. The rats given 6.6 g/kg ethanol had high peak blood ethanol concentrations (mean=353 mg/dl). Rats in the 3.3 g/kg group had significantly lower peak blood ethanol concentrations (mean=152 mg/dl). On PD 10 all rats were perfused, the brains extracted, postfixed and the cerebellar vermis isolated and embedded in JB-4. Purkinje cells were counted from 2 mm thick midsagittal sections stained with cresyl violet/saffronin O. Suckle and gastrostomy controls did not differ significantly in the overall number of Purkinje cells. The rats given 6.6 g/kg ethanol had significant reductions in Purkinje cells compared to all other groups ($p<.01$). Additional analysis revealed significant reductions in lobules 1-5 and 9-10 but not in lobules 6-8. The pups exposed to 3.3 g/kg ethanol demonstrated significant reductions in overall number of Purkinje cells compared to suckle controls but not compared to gastrostomy controls. The Purkinje cell loss in this study implies that a brief exposure to a relatively high blood alcohol level during the brain growth spurt, as may occur in "binge" drinking during the third trimester, may constitute an increased risk to vulnerable cell populations in the developing central nervous system. Supported by grant AA05523 to JRW.

408.9

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

A rat model of third trimester alcohol administration was used to examine differences in vulnerability to alcohol-induced cell loss among neuronal populations. Sprague-Dawley rat pups were reared artificially over postnatal days 4-10 (a period of rapid brain growth similar to the human third trimester). Alcohol-exposed pups received 4.5 g/kg/day ethanol in either 2 or 4 of the 12 daily feedings (administered as 10.2% or 5.1% v/v solutions, respectively). Controls included non-alcohol exposed gastrostomy controls and normally-reared suckle controls. On postnatal day 10, the pups were perfused with fixative, and the brains were removed. Two-micron-thick sections were cut horizontally through the midtemporal hippocampal formation, sagittally through the midline cerebellar vermis and coronally through the mid-olfactory bulb. From single sections, the following cell populations were counted: hippocampal neurons in fields CA1, CA2/3 and CA4, dentate gyrus granule cells, cerebellar Purkinje cells and olfactory bulb mitral cells. Cerebellar granule cells were estimated. Neuronal populations were not affected equally by the alcohol treatments. In the hippocampus, there was a significant loss only of CA1 pyramidal cells, and this reduction occurred only in the group receiving the high concentration (10.2%) solution. Cerebellar Purkinje cells and olfactory bulb mitral cells were significantly reduced in both alcohol-treated groups. Cerebellar granule cells were significantly reduced, but dentate gyrus granule cells were unaffected. These result demonstrate that neuronal populations differ considerably in their vulnerability to alcohol during development. (Supported by NIAAA grant AA05523 to J.R.W.)

408.10

EFFECTS OF MATERNAL ETHANOL CONSUMPTION PRIOR TO AND DURING GESTATION ON THE SPINAL CORD OF THE NEONATAL RAT. D.L. McNeill, E.L. Fagan*, C.H. Harris* and R.L. Shew*. Dept. of Anat. Sci., Univ. of Oklahoma, Oklahoma City, OK 73190

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

408.11

DISTRIBUTION OF CALLOSAL PROJECTION NEURONS IN SOMATOSENSORY CORTEX OF RATS PRENATALLY EXPOSED TO ETHANOL. L.A. Kotkoskie* and M.W. Miller (SPON: R. Rieck). UMDNJ- Sch. Osteo. Med. & R.W. Johnson Med. Sch., Piscataway NJ 08854.

Prenatal ethanol exposure produces permanent laminar and cellular defects in neocortex. These malformations have been attributed to alterations in early developmental processes such as neuronal migration and differentiation. The present study examined the effect of gestational ethanol exposure on the laminar distribution of callosal projection neurons in mature rat neocortex. The subjects, 3-6 month old hooded rats, were the progeny of mothers prenatally fed a liquid diet containing 6.7% (v/v) ethanol, an isocaloric liquid control diet, or a chow diet. Horseradish peroxidase (HRP) was injected unilaterally into area 3 of primary somatosensory cortex. After a post-injection survival period of 2 days, each brain was processed for HRP histochemistry and stained with cresyl violet to identify laminar patterns. In control animals, callosal projection neurons were distributed in layers II-VI of the contralateral somatosensory cortex, but most HRP-positive neurons were in layers II/III and V of somatosensory cortex. In contrast, about 75% of the callosal projection neurons in ethanol exposed animals were in layers V and VI. We hypothesize that gestational ethanol exposure produces an abnormal distribution of callosal projection neurons in somatosensory cortex from the ectopic placement of cortical neurons or the abnormal differentiation of infragranular cortical neurons. Funded by AA 06916, AA 07568, DE 07734 and NS 07229.

409.1

MICRODIALYSIS,IN THE CISTERNA MAGNA,OF Na TRANSPORT INTO THE CEREBROSPINAL FLUID (CSF) SYSTEM. C.E. Johanson, N. Knuckey and A. Fowler*. Program in Neurosurgery, Dept. of Clin. Neurosciences, Brown Univer./ R. I. Hospital, Providence, RI 02902.

Microdialysis in vivo has been used to study extracellular compounds in CNS. This report describes intracranial dialysis to analyze CSF dynamics in rats. The choroid plexus is the major source of CSF, which is produced by active transport of Na from blood to ventricles. By injecting Na-22 into peripheral circulation and measuring its movement into CSF by microdialysis, one should obtain a measure of the rate of CSF formation.

Adult, Sprague-Dawley rats (200-300 g), anesthetized with ketamine and xylazine, were kept at 37 C while being prepared for implantation of the probe (CMA/10 Microdialysis Probe, Carnegie Medicin). In vitro probe efficiency for Na was 15%. Following arterial cannulation for MABP and plasma Na-22 analysis, the occipital skull plate and dural membrane over the cistern were exposed. A small hole was drilled at the external occipital crest. The probe, mounted on a stereotaxic holder, was visible through the membrane after its insertion. Agar gel around the hole prevented CSF leakage. The dialysis pump (CMA/100 Microinjection, Carnegie), containing artificial CSF, was turned on at 2 ul/min. Plasma turnover of Na-22 into the dialysate was reduced 45% by acetazolamide (25 mg/kg). Cisternal dialysis shows promise for testing drug effects on CSF.

409.2

DEVELOPMENT OF GLUCOSE TRANSPORTER IN BRAIN:IMMUNOCYTOCHEMISTRY AND IN SITU HYBRIDIZATION OF mRNA. A.M. Morin, D.S. Thomson*, K. CLegg* & H.V. Vinters*. Neurology Res. Lab. VA Med Center, Sepulveda, CA 91343 and Depts. Neurol. & Neuropath. and Brain Res. Inst. UCLA School of Med. Los Angeles, CA 90024.

The glucose transporter (GT) promotes the entry of glucose into brain through the blood brain barrier by means of facilitated diffusion. A previous description of the development of the transporter (Morin et al., J. Neurochem. 51(1)206-211, 1988) indicates that it is present in rat brain and enriched in cerebral microvessels as early as 4-7 days post-natal. The present work describes the appearance of GT detected with immunohistochemical staining and mRNA for the GT detected via in situ hybridization. At 4 days post-natal, capillaries can be seen to stain with antibody while immunoreactive material is seen in cell bodies adjacent to the capillaries. This "halo" of immunoreactivity is seen predominately in frozen, sectioned neonate brains and is relatively absent in adult brains. Results of in situ hybridization indicate the presence of mRNA transcripts in many cells as early as 4 days post-natal. This work supported by the Research Service of the Veterans Administration and a grant (BRSG) from the Neuropsychiatric Inst., UCLA.

409.3

CEREBROSPINAL FLUID (CSF) LEVELS OF MG DURING IV INFUSION OF $MGCL_2$ IN DEVELOPING SWINE. L.I. Rivera*, R-H. Lin*, P.M. Gootman, H.L. Cohen, N. Gootman, Div. Pediatr. Cardiol. Schneider Child. Hosp., LIJMC, A. Einstein Col. Med., New Hyde Park, NY, 11042 and Dept. Physiol. SUNY-Hlth. Sci. Ctr. Bklyn., Bklyn, NY 11203

It has long been thought that Mg does not pass the blood brain barrier (BBB) (Bradbury, 1979 for review) but rather that it's actions are all peripheral. Nervous system responses to changes in plasma [Mg] have been used as the example of the efficacy of Mg homeostasis in cerebral extracellular fluids. Further, it has been thought that by birth the BBB is intact for Mg. We decided to re-examine these results in light of observations made in developing swine to increasing plasma levels of Mg (Gootman, et al. Neurosci. Abst. 1988, 14:415; Rivera et al.FASEB J. 1989, 3:A1070). 0.2M $MgCl_2$ was infused for 1 hr and arterial plasma samples drawn at 10 min intervals. Aortic pressure, EKG, end-tidal CO_2 and intratracheal pressure were continuously monitored and blood gases and pH were measured at @ 45 min intervals. CSF was obtained either at 10 min intervals or prior to and at end of infusion. Parallel increases of [Mg] occurred in both plasma and CSF to @ 13 mg/dl; beyond this concentration, plasma levels continued to rise while CSF levels stablized. CSF [Mg] rose from 3.0±.35 to 13.4±1.5 mg/dl ($p < .01$). Our results indicate that Mg can pass the BBB as plasma levels of Mg rise, thus paralleling the increase in plasma concentration. (Support-NIH grant HL-20864.)

409.4

STRUCTURAL SPECIFICITY OF THE BRAIN CAPILLARY NEUTRAL AMINO ACID TRANSPORTER. Quentin R. Smith, M. Aoyagi*, and S. I. Rapoport. Lab. of Neurosciences, NIA, NIH, Bethesda, MD 20892.

Neutral amino acids are transported from plasma into brain by a high affinity, saturable mechanism at the brain capillary endothelium. This carrier is known to facilitate the brain uptake of several drugs (L-dopa, α-methyldopa, melphalan) and may provide a means of enhancing brain delivery of other CNS therapeutic agents. To characterize the structural requirements of the transporter, the ability of over 100 solutes to inhibit L-[14C]-phenylalanine uptake into brain was determined in anesthetized rats using the in situ brain perfusion technique of Takasato et al. (1984). Both a free carboxyl group and an unsubstituted α-amino group were found to be required for strong binding to the carrier. Amino acids with large side chains, such as α-aminononanoic acid and O-benzyl-L-tyrosine, bound readily, indicating minimal limitation of amino acid size. Affinity depended critically over a 10,000-fold range on side chain hydrophobicity. For a series of straight chain alkyl amino acids, the calculated ΔG for binding fell from ~1 kcal/mol/CH_2 for the first three CH_2 groups to 0.2-0.5 kcal/mol/CH_2 for the next four CH_2 groups, indicating interaction with a strongly hydrophobic region. The results suggest that the brain capillary neutral amino acid carrier can accept a wide variety of ligands and therefore may be useful in facilitating drug entry into the CNS.

409.5

NEUROHUMORAL REGULATION OF AMINO ACID TRANSPORT IN RAT CEREBRAL MICROVESSELS. P. Grammas*, T.M. Kwaiser* and M.L. Caspers. Dept. of Pathology, Wayne State Univ. Med. School, Detroit, MI 48201 and Dept. of Chemistry, Univ. of Detroit, Detroit, MI 48221.

Enzymatic functions of the blood-brain barrier (BBB) involved in amino acid transport i.e. γ-glutamyltranspeptidase are modulated by adrenergic and cholinergic receptors. In the present study, uptake of ^{14}C-α-methylaminoisobutyric acid (MeAIB), a nonhydrolyzable analog used to probe A-system transport, was examined under basal and agonist-stimulated conditions. Nonspecific binding was determined in the presence of 1 mM ouabain. The specific uptake of MeAIB at 37°C was time-dependent and reached a maximum at approximately 30 min. Neurohumoral control of amino acid uptake was explored using the α and β-adrenergic agonists phenylephrine (PE) and isoproterenol (ISO) and the muscarinic agonist carbachol (CA). Cerebral cortical microvessels incubated with 10 μM PE or 10 μM ISO demonstrated an increase in the uptake of MeAIB of 109 and 111% over basal, respectively. While 10 μM CA alone did not alter the uptake of MeAIB by microvessels, it blocked the stimulation of uptake evoked by adrenergic agonists. These data suggest that carrier-mediated transport of amino acids across the BBB are under dual adrenergic-cholinergic control. Supported in part by PHS HL 23603, ADRDA, U. Detroit faculty award and J.D. Rose.

409.6

BLOOD-BRAIN BARRIER [BBB] TO PEPTIDE ENTRY IN VITRO. B.R. Brooks, T. Schwartz*, G. Kaminska*, J. Turner* and J.M. Rozental*. Neurology Dept, Univ of Wisconsin Med Sch and Wm S Middleton VA Hospital, Madison, WI 53705

Peptide entry into the CNS requires transport without chemical modification through the BBB consisting of the endothelial cell [EC] and astrocyte [A]. Degradation of [^{3}H-pro] thyrotrotropin releasing hormone [TRH] was studied in three separate cultures of purified ECs from the brain [Br] or spinal [SC] of Balb/c mice. Media samples were collected at 0, 10, 20, 30 and 60 minutes following incubation at 37°C. TRH degradation measured by HPLC [J Chromatog 487:275,1989] was signficantly [$p < 0.05$] elevated in Br As [95 ± 57 (SD) femtomole/mg protein/min] and SC As [54 ± 2] compared with Br ECs [14 ± 11]. Cyclic histidyl-proline formation was signficantly [$p < 0.05$] increased in both Br [2030 ± 1435] and SC [1380 ± 153] As compared with ECs [165 ± 285]. Comparable product increases were seen also for TRH-OH and $ProNH_2$ but not Pro. Scraped cells contained essentially no labelled products. Surface peptidases are present on ECs, but the specific activity against a tri-peptide is 10 X increased on the surface of Br and SC As. These peptidases limit the spread of active peptides after diffusion across the ECs or through the interstitial space after intrathecal administration. **[Supported by MDA Midwest ALS Center]**

409.7

DIFFERENTIAL GROWTH RATE OF BALB/C BRAIN AND NON-BRAIN ENDOTHELIAL CELLS [EC] PREPARED BY DEXTRAN-PERCOLL STEP GRADIENTS. J.M. Rozental*, D. Schrader*, S. Scheibengraber*, and B.R. Brooks. (SPON: R.Daly) Neurology Dept, Univ of Wisconsin Med Sch and Wm S Middleton VA Hosp, Madison, WI 53705.

The growth potential in vitro of primary EC from different organs in 10 d Balb/c mice was studied. Rapid purification of vascular EC from cerebral cortex [CTX], subcortex [sCTX], cerebellum [CBL], spinal cord [SC], kidney [KID], lung [LUN], liver [LIV] and spleen [SPL] used an enzyme digestion, Dextran-Percoll step gradient technique. Aorta [A] was prepared without step gradients. Tissues were homogenized in 0.5% collagenase-0.2% DNAase. The first gradient through 15% dextran yields neurons and glia at the top and vascular stroma contaminated by rbc and wbc in the pellet. The pellet is resuspended for a second gradient in 45% Percoll. A monodisperse EC band just touches the upper margin of a microvessel remnant band. Viable cell recoveries are different from brain and non-brain organs. CTX, sCTX, CBL, and KID EC grow out equally well on fibronectin and gelatin while fibronectin is essential for A, LUN, LIV and SPL EC. Cultured EC are von Willebrand Antigen [Factor VIII], alkaline phosphatase [+] and GFAP, UEA-lectin [-]. A EC grow faster in vitro than non-brain and brain EC. The growth rate of CTX and CBL EC is faster than that of sCTX and SC EC. (Supported by VA Res Svc)

409.8

EFFECTS OF POLYMORPHONUCLEAR CELLS (PMNs) ON THE FORMATION OF EICOSANOIDS AND KININS BY A MURINE ENDOTHELIAL CELL (CEC) LINE. J. Xu*, J. Chao*, S.A. Moore, E.L. Hogan, C.Y. Hsu. Dept. of Neurology, Medical University of South Carolina, Charleston, SC 29425 and Dept. of Pathology, Univ. of Iowa, Iowa City, IA 52242.

Inflammatory mediators including eicosanoids and bradykinin have been implicated in the pathogenesis of tissue injury and edema formation following ischemic or traumatic CNS insults. Interaction of CEC with inflammatory cells may contribute to this process. Using a CEC line (Moore et al, Am. J. Physiol 254 C37, 1988) we studied the effect of PMNs on A23187-stimulated conversion of kininogen to kinin and formation of leukotriene B_4 (LTB_4), thromboxane B_2 (TXB_2) and 6-keto-PGF1α (6KF). Table shows concentrations of mediators in serum-free medium following incubation of confluent CEC (0.22 mg protein/well) with or without PMNs (10^6 cells/ml). Purified kininogen (2 μg/ml) and A23187 (10 μM) were added as indicated. Data shown are mean $\pm$ SE from 3 experiments of triplicate wells

Exp. Conditions			Kinin	LTB_4	TXB_2	6KF
PMN	CEC	A23187	(pg/ml)		(ng/ml)	
-	+	+	21 $\pm$ 2	-	-	82 $\pm$ 4
+	-	+	46 $\pm$ 12	36 $\pm$ 2	27 $\pm$ 4	-
+	+	-	47 $\pm$ 18	-	6 $\pm$ 1	24 $\pm$ 2
+	+	+	87 $\pm$ 17	48 $\pm$ 3	26 $\pm$ 3	110 $\pm$ 5

Results indicate that PMN-CEC interaction may enhance the formation of kinins, LTB_4 and 6KF but not TXB_2.

409.9

A PROCEDURE FOR THE ISOLATION AND CULTURE OF ADULT RAT BRAIN ENDOTHELIAL CELL-CONTAINING MICROVESSEL FRAGMENTS (ECCMF). D.A. Doron*, D.M. Jacobowitz, G. Feuerstein, H.B. Pollard and J.M. Hallenbeck*. U.S.U.H.S., Bethesda, MD 20814 and Lab. Clin. Sci., NIMH, Bethesda, MD 20892.

A number of laboratories have found the culture of endothelial cells from adult rat brain microvessels (MV) difficult to achieve. We therefore proceeded to develop a procedure for the isolation and culture of small ECCMF. We followed traditional methods for isolation of MV. The vessels were purified through a dextran/percoll gradient and a nylon mesh was used to isolate the microvessel fraction. ECCMF were plated on fibronectin or gelatin coated dishes. Although growth of cells were observed, they did not stain for Factor VIII related antigen (FVIIIag). However, when the ECCMF were plated on Matrigel (basement membrane collagen), positive staining for FVIIIag, transferrin receptor and incorporation of low density lipoprotein was observed. Initial studies were undertaken to test the viability of this preparation. Prostacyclin (PGI2) was measured after 24 hrs of equilibration as 6-Keto PGF1α by a RIA. ECCMF maintained in media containing 20% FCS produced 5.89 ng/ml after 10 min. Incubation with bradykinin, thrombin and ionomycin induced a significant increase of PGI2 production (104%, 223% and 88% respectively). It appears that this preparation will be useful for further studies on the effect of a variety of substances which are potentially active at the MV level.

409.10

ISOLATED CEREBRAL MICROVESSEL (ICMV) ENERGY STATE: ROLES OF GLYCOLYSIS & LIPID OXIDATION. A. McCall and C. Gaposchkin*. Depts. of Med. & Physiol., B.U. Sch. Med.,Boston, MA 02118

ICMV can undergo a "metabolic shock" indicated by low initial ATP levels and ATP/ADP ratios that recover on incubation in enriched medium. To determine which fuels best maintain microvessel energy state, we incubated ICMV with single metabolic fuels and measured their ATP and ADP contents hourly using a bio-luminescent assay. Below are ATP/ADP ratios for ICMV incubated fuel-free or in glucose.

Incubation	0h	1h	2h	3h	4h
Fuel-Free (x $\pm$SE)	3.2 $\pm$0.2	2.5 $\pm$0.3	2.1 $\pm$0.1	1.8 $\pm$0.1	1.6 $\pm$0.3
Glucose (5.5 mM)	---	3.8 $\pm$0.5	4.9 $\pm$0.4	5.0 $\pm$0.5	4.7 $\pm$0.6

The improvement with glucose was not seen with either pyruvate (2.0 mM), 3-hydroxybutyrate (2.0 mM), or glutamate (2.0 mM). Oleate (0.33 mM) increased microvessel ATP/ADP ratios slightly only when co-incubated with carnitine. Incubation with 2-tetradecylglycidate, a specific inhibitor of long chain fatty acid oxidation, further reduced the ATP/ADP ratio in fuel-free incubation by 75%; the effect was countered by glucose. Conclusions: Increases in ICMV ATP/ADP ratios with glucose but not pyruvate suggest that glycolysis is a major source of ATP for ICMV. However, oxidation of endogenous fatty acids may make a significant contribution in fuel deprived ICMV. This dependence on glycolysis might result from ischemia during isolation.

409.11

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

We tested the effect of several putative vasoactive substances, mostly peptides for which there is evidence that their receptors exist in brain microvessels, for their effect on cyclic GMP generation in isolated rat brain microvessels. Bulk isolated rat cerebral microvessels were incubated with atrial natriuretic peptide (ANP), 10^{-9} to 10^{-5}M; angiotensin II, 10^{-6} to 10^{-5}M; arginine vasopressin, 10^{-6} to 10^{-5}M; bradykinin, 10^{-6}M; carbachol, 10^{-4} to 10^{-3}M; or thrombin, 10^{-6} to 10^{-4}M for a period of 10 min after which the reaction was stopped and the microvessels were extracted with perchloric acid and the content of cyclic GMP in the extract was assayed by radioimmunoassay. In all experiments, the ability of sodium nitroprusside (10^{-5}) to increase guanylate cyclase activity was ascertained. Of all the agents studied, only ANP was potent in stimulating guanylate cyclase. This stimulation was dose dependent, with maximum stimulation at 10^{-6}M. Our results indicate that ANP receptor stimulation in brain microvessels is mediated, at least in part, by cyclic GMP. Receptors for the other vasoactive substances that were tested, if they exist in rat cerebral microvessels, are not related to cyclic GMP.

410.1

DEFICIENT NEURITE OUTGROWTH OF RETINAL EXPLANTS FROM ADULT MOUSE ON ASTROCYTES. R.L. Meyer, J. Miotke* and J. Fawcett. Dev. Biol. Center, Univ. Calif., Irvine, CA 92717 and Dept. Physiol., Cambridge Univ., Cambridge, UK CB2 3EG.

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

In this study, adult and embryonic retinal explants were made on astrocytes which were made from 1-2d old mouse and maintained in culture 8d to 7wk. In some preparations oligodendrocytes were removed by shaking or by anti-GalC plus complement. Neurites were visualized by anti-neurofilament immunohistochemistry. At 1wk, adult retinal explants had fewer and shorter neurites on these cellular substrates compared to laminin. In contrast, embryonic retina (E-15) sent out numerous long neurites on these same cellular substrate as early as 2d even when numerous oligodendrocytes were present.

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

410.2

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

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

410.3

MONOCLONAL ANTIBODY 8A2 TRIGGERS EVACUATION OF GROWTH CONE CONTENTS AND DISTO-PROXIMAL BULK REDISTRIBUTION OF AXOPLASM IN GROWING AXONS. S. Finnegan Sloan*, E. Koenig and V. Lemmon. Dept. of Physiology, SUNY at Buffalo, Buffalo, N.Y., and Center for Neurosciences, Case Western Reserve Univ., Cleveland, OH 44106.

Monoclonal antibody 8A2 has been shown to recognize a class of O-acetylated ganglioside and to inhibit neurite outgrowth of chick *in vitro* (Soc. Neurosci. Abstr. Vol. 14, p. 272). Phase-contrast video-microscopic studies reveal that 8A2 (1:1000) induces a global evacuation of the contents of growth cones in regenerating retinal ganglion cell axons of goldfish explants *in vitro*. The onset of effects is rapid and signaled by an immediate cessation of elongation, a loss of lamellipodia and an emergence of a prominent filopodial morphology. A retrieval of axoplasm begins in distal filopodia, and the material accummulates at the base of the growth cone. With time, there is an *en masse* redistribution of accumulated axoplasm in the retrograde direction, such that distal evacuated segments measure 30-80 μm after an hour. Membrane filopodial ghosts mark the original site of attachment. D1.1 monoclonal antibody, which recognizes O-acetylated GD_3 ganglioside (courtesy of Dr. Joel Levine), does not induce a global disto-proximal evacuation. Fluorescence microscopy shows very strong 8A2 and D1.1 immunofluorescence distributed uniformly over the surfaces of axons. After treatment of living axons with 8A2, followed by Triton X-100 extraction, immunofluorescence is punctate, indicating an apparent stabilization of some antigen. Chloroform-methanol extraction of fixed axons appears to remove most, but not all immunofluorescence.

410.4

A MONOCLONAL ANTIBODY WHICH RECOGNIZES GROWING AXONS IN THE GOLDFISH VISUAL SYSTEM J. Vielmetter and C.A.O. Stuermer Friedrich-Miescher-Laboratorium der Max-Planck-Gesellschaft 7400 Tübingen FRG

During the continuous growth of the goldfish retinotectal system, new ganglion cells are added at the peripheral retinal margin. The axons of these cells travel as a coherent, age related bundle through the optic nerve and tract (Easter et al. '81) and around the dorsal and ventral margin of the tectum (Stuermer and Easter '84). These young axons are specifically recognized by a new monoclonal antibody, ϵ587.

This antibody was obtained by immunizing mice with glycoproteins extracted from cell surface membranes of adult goldfish tecta and purified with lentil lectin and WGA affinity chromatography. On frozen sections ϵ587 stains axon bundles in locations identical to the position of newly added ganglion cell axons. After optic nerve section, the retinal axons regenerate. Most or all regenerating axons are ϵ587 positive in the nerve distal to the cut, in the tract, and in the retinorecipient layers, SO and SFGS, of the tectum. Unfixed axons *in vitro* emerging from retinal explants and growing on laminin, are stained by ϵ587 throughout their length including their growth cones and filopodia. Staining with ϵ587 does not require fixation or permeabilization of the cell membrane, indicating that the antigen is exposed on the cell surface. On western blots ϵ587 recognizes a protein of an apparent molecular weight of 200 kd. The antigen therefore is different from the antigen recognized by α-N-CAM (Bastmeyer et al. '89).

Thus the ϵ587 antigen is a cell surface protein which appears to be specific for growing retinal axons in fish.

410.5

MONOCLONAL ANTIBODY M802 RECOGNIZES REGENERATING RETINAL AXONS IN GOLDFISH K.A.Wehner and C.A.O.Stuermer, (Spon: A.D. de Lima). Friedrich-Miescher-Laboratorium der Max-Planck-Gesellschaft, Tübingen, FRG.

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

On frozen sections of normal fish, M802 staining is restricted to bloodvessels. However, in fish with previously transected optic nerves, the retinal fiber layer in the eye, the optic nerve and tract and the retinorecipient layers of the tectum are labeled intensively. M802 staining in these regions appears to persist for more than six months after ONS but has decreased in intensity by 9 months.

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

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

410.6

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

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

The tPoC intersects with three other tracts. At these intersections, the morphologies and trajectories of axons entering the tPoC change abruptly and we have interpreted these changes in the context of axonal pathfinding. Supported by NATO (BRF8217) and NIH (EY-00168).

410.7

DEVELOPMENT OF FIN NERVES IN WILD-TYPE AND MUTANT FISH EMBRYOS. H. Okamoto* and J.Y. Kuwada. (SPON: B. Oakley). Dept. of Biology and Gerontology, U. of Michigan, Ann Arbor, MI 48109.

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

410.8

PATHFINDING BY IDENTIFIED NEURONS IN THE BRAIN OF ZEBRAFISH EMBRYOS. A.B. Chitnis* and J.Y. Kuwada (SPON: D. Rosenblatt). Dept. of Biology and Gerontology, U. of Michigan, Ann Arbor, MI 48109

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

410.9

PATHFINDING BY SPINAL GROWTH CONES IN THE ZEBRAFISH EMBRYO. J.Y. Kuwada, R.R. Bernhardt, A.B. Chitnis*, and N. Nguyen*. Dept. of Biology and Gerontology, U. of Michigan, Ann Arbor, MI 48109.

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

410.10

NEURONS IN THE DEVELOPING ZEBRAFISH SPINAL CORD. R.R. Bernhardt and J.Y. Kuwada. Dept. of Biology and Gerontology, U. of Michigan, Ann Arbor, MI 48109.

As a basis for an analysis of axonal pathfinding we have characterized the cellular anatomy of the simple spinal cord of developing zebrafish with intra- and extracellular dye injections and various antibodies. Nine classes of embryonic neurons have been distinguished based on soma postion, axonal trajectory, and time of axonogenesis (Co=commissural, Ci=circumferential, L=longitudinal, A=ascending, D=descending). There are 3 classes of Co-cells; early primary (CoPA), later secondary (CoSA), and bifurcating (CoB). Ci-cells are similar to Co-cells but have ipsilateral axons and fall into 2 classes (CiA and CiD). The L-cells consist of dorsal (DLA) and ventral (VLD) cells. Also there are Rohon-Beard (RB) and motor neurons. These cell types account for 50-75% of the 50-80 neurons per embryonic spinal segment. RBs and VLDs pioneer an early dorsal (DLF) and ventral (VLF) fiber tract, respectively. CoPAs establish the commissural pathway. Most of the embryonic cell types are also found in larvae (3-5 d). Larval CoPAs and RBs project to supraspinal levels (di-/mesencephalon and hindbrain, respectively). EM shows that the DLF and VLF are established by a small number of axons at 16-20 h. At this stage only scattered axonal profiles are found in the imtermediate lateral margin (presumably Co-axons). At 25-40 h addition of new axons, both to preexisting bundles and at intermediate levels, creates a continuous marginal zone. Reticulospinal axons establish a distinct ventral medial bundle (MLF) and commissural tracts are evident at 2-4 d. Supported by grants from NIH, March of Dimes, and OVPR at UM.

410.11

MOTOR INNERVATION OF DORSOVENTRALLY REVERSED WINGS IN CHICK/QUAIL CHIMAERIC EMBRYOS. M. Ferns* & M. Hollyday. (SPON: E. Thomas). Dept. of Biology, Bryn Mawr College, Bryn Mawr, PA 19010.

During the development of motor innervation of the chick limb, lateral and medially positioned motoneurons within the lateral motor column (LMC) have been shown to project selectively to muscles of dorsal and ventral origin respectively. We have examined the axonal guidance cues involved in this initial, specific pathway choice at the plexus by making dorsoventral (D/V) limb-bud reversals prior to innervation. Chick wing buds were surgically removed and replaced by quail in D/V reversed orientation at St 18, producing chimaeric embryos in which the level of the reversal could be determined. Specificity of the innervation of the reversed wing was assessed at St 28-29, prior to the onset of motoneuron death, by retrograde HRP labelling of either the dorsal radial or ventral brachialis inferior nerve trunks.

The ability of axons to correct for the reversal was found to be dependent on the level of the graft being proximal to the plexus, and on the reversed limb and gross nerve pattern being normal, particularly in the shoulder region. In such cases (n=8), after normal dorsoventral sorting in the spinal nerves, many motor axons clearly altered their trajectories in the graft tissue, at varying levels within the donor plexus region, and projected appropriately for their position in the LMC. The D/V pathway cues therefore are unlikely to be long range target derived signals, but rather appear to be closely associated with positional information in the plexus region (and perhaps more proximally). The correlation between active correction and the development of normal limb pattern leads us to suggest that axons may respond, in a specific fashion, directly to the positional information signal encoding the D/V axis, or indirectly to cues which differentiate in accord with D/V positional value.

(Supported by NIH NS-25340).

410.12

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

The initial pattern of innervation of the chick limb has been reported to be highly specific, with only few instances of segmentally erroneous projections being detected. One possible form of projection error however, which has received little attention, is collateral branching of axons to divergent targets.

Axons projecting in either the dorsal radial or ventral brachialis inferior nerve trunks in the wing were retrogradely labelled by direct HRP injections at St 28-29, shortly after the formation of muscle nerve branches, and detected immunohistochemically in paraffin sections with the sensitive peroxidase anti-peroxidase technique. Collaterals of retrogradely labelled axons were consistently observed to project into the subset of muscle nerves diverging from the nerve trunk proximal to the injection site. Collaterals were present in moderate numbers as compared to the number of labelled axons and extended varying, but often considerable distances (200-500μm) into the muscle nerves. Collateral branching at the plexus where the pathways to dorsal and ventral muscle masses diverge was only rarely observed. Therefore, whereas axonal branching at the muscle nerve branches may reflect localized regions of increased (but specific) substratum adhesivity, differing guidance mechanisms are perhaps operative at the dorsoventral pathway choice.

It remains to be determined whether such projection of collaterals to multiple targets involves motor and/or sensory axons, and what is the time course and mechanism of their presumed selective elimination. It appears however that axonal guidance mechanisms are, in some instances, of limited accuracy and resolution, producing some imprecision in the initial pattern of projections.

(Supported by NIH NS-25340).

410.13

HNK-1 LABELS DEVELOPING XENOPUS NEURONS AND GROWTH CONES. R.H.Nordlander. Dept. Oral Biol., Case West. Reserve Univ. Sch. Dent. Cleveland, OH 44106.

The monoclonal antibody HNK-1 has been widely used to identify migrating neural crest cells. The present study examines the expression of the HNK-1 epitope in the developing nervous system of Xenopus embryos and larvae as viewed in wholemounts. In these preparations HNK-1 gives a readily accessible overview of the developing fiber scaffolding of the nervous system much like that provided by anti-HRP marking of the earliest neural elements of Drosophila.

The antibody was found to mark developing neurons from the time of initial axonal outgrowth. It appears on the surfaces of cell bodies, axons, and axonal growth cones of identifiable neurons. Marking is transient and its distribution follows a rostrocaudal developmental gradient in a pattern consistent with observations from earlier studies using neuronal tracers and electron microscopy.

The association of this epitope with the surfaces of developing neurons suggests a function in axonal outgrowth.

The author thanks E.Jaszczak for technical assistance and S.Landis for HNK-1. This work was supported by NS-18873.

410.14

COCULTURES OF MERKEL CELLS, SENSORY NEURONS & SYMPATHETIC NEURONS IN SERUM-FREE MEDIUM. P. Vos & R.N. Pittman, U. of PA., Sch. of Med., Dept. of Pharmacol., Phila. PA, 19104

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

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

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

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

410.15

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

Axial cutaneous nerves emerge from the sensory ganglia several days after their counterparts that innervate cutaneous targets in the chick hindlimb. Despite the temporal differences in initial outgrowth, both axial and limb cutaneous nerves grow from nerve trunks toward the epidermis at the same time (stage 26.5) as though both were responding to a general maturation of their target. To test this notion, I am replacing chick epidermis with older or younger epidermis from quail and determining whether subsequent outgrowth of axial cutaneous nerves is proportionally advanced or delayed in time.

Preliminary results indicate that the formation of axial cutaneous nerves is temporally correlated with the maturity of the epidermis. Cutaneous nerves will form before stage 26 if their normal target is replaced with older epidermis. This stimulation of outgrowth is not likely to be an artifactual result, since younger target can suppress outgrowth. In addition, a small lateral patch of more mature epidermis can elicit formation of a novel cutaneous branch from the distal spinal nerve. The mature epidermis stimulates outgrowth despite the fact that it is usually well beyond filopodial reach. These results are consistent with a relatively long-distance cue emanating from the epidermis that attracts sensory growth cones. Alternatively, the epidermis may change or condition the more proximal pathway, rendering it more suitable for advance. These preliminary results provide the first experimental evidence that temporal alterations in the target may contribute to the control of axonal outgrowth.

Supported by NIH grant #NS-21308.

410.16

DEVELOPMENT AND INNERVATION OF THE PRIMARY ABDOMINAL MUSCLE IN EMBRYONIC *XENOPUS LAEVIS*. K.L. Lynch* and S.E. Fraser. (SPON: R. Lewis) Dept. of Anat., USUHS, Bethesda, MD 20814-4799 and Dept. of Physiol. & Biophysics, University of California, Irvine, CA 92717

The primary abdominal muscle of *Xenopus* develops during embryonic stages 31 to 40, from cell clusters that bud from the ventrolateral margins of trunk myotomes II to VIII. Because of its thin flat shape, regular segmentation and superficial position, the abdominal muscle is an ideal subject for studies in *vivo* of cell migration, muscle morphogenesis and differentiation, and interactions between myogenic cells, extracellular matrix components, and the nerve fibers that innervate the muscle.

The undifferentiated cells from which the abdominal muscle develops migrate ventrally from the myotomes as clusters of tightly adherent cells, a relatively unusual form of cell migration. We have used the fluorescent tracer DiI to label the clusters and record their movement by time-lapse videomicroscopy. The tracer also enabled us to identify the most rostral of the myotomal buds as the source of the muscle fibers in the geniohyoid muscle. Silver stains, electron microscopy and enzyme histochemistry revealed that the first motor fibers to the abdominal muscle, from the ventral rami of spinal nerves 2 through 9, initially follow the paths of the cell clusters from which the muscle develops. When the cells of the clusters differentiate and fuse to form myotubes, however, the motor axons are in trunks located deep to the narrow zones between adjacent muscle segments. Fibers from spinal nerve 2 innervate the first segment of the abdominal muscle and the geniohyoid, consistent with the origin of the myogenic cells.

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

410.17

POLYSIALIC ACID AS A REGULATOR OF NERVE BRANCHING IN DEVELOPING CHICK MUSCLE. L.T. Landmesser, L.M. Dahm, U. Rutishauser, and J. Tang*. Dept. Physiol. & Neurobiol., Univ. of CT, Storrs, CT 06269 and Case Western Reserve Univ. Sch. Medicine, Cleveland, OH 44106.

The polysialic acid (PSA) moiety of NCAM can regulate the strength of cell-cell interactions mediated by either NCAM or by G4/L1. We previously found that the strength of axon-axon adhesion due primarily to G4/L1 has a strong influence on the degree and spatial pattern of nerve branching in embryonic muscle. Further, in-ovo inactivation of G4/L1 with specific antibodies produced changes in the nerve pattern similar to those following dTC-induced neuromuscular activity blockade. We report here that activity block does not decrease the actual level of G4/L1; however, it does cause a large increase in PSA levels in nerve, which we propose indirectly decreases G4/L1 function. This increase in nerve PSA can account for most of the inactivity-induced changes in branching, since PSA removal via an endoneuraminidase prevents these changes in dTC-treated embryos. Since antibodies to G4/L1 but not to NCAM effectively reversed the changes in branching produced by PSA removal, we conclude that G4/L1 is the adhesive ligand most responsible for the PSA-regulated changes in nerve branching. During normal development, PSA levels in nerve were also regulated in a temporal-spatial pattern consistent with its postulated role in nerve branching.

Supported by NIH grants NS 19640 and HD 18369.

410.18

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

We are interested in elucidating the mechanisms by which sensory neurons (SNs) grow to their correct targets in the chick hindlimb during development. We are currently investigating how interactions of SN growth cones with axons they normally encounter may influence their pattern of outgrowth, using an in vitro model system (Honig, Neurosci. Abstr., 13: 1220, 1987). Here we report our results on the interactions of SN growth cones with the processes of other SNs in dissociated cell cultures. We have seen a variety of behaviors when we observe these encounters using time-lapse videotaping. As expected, based on work by Kapfhammer and Raper (J. Neurosci., 7: 1595, 1987), the most common response was crossing. This was often, but not always, preceeded by a delay during which the growth cone maintained contact with the neurite. Somewhat unexpectedly, we have also seen other types of responses. In some cases, the growth cone fasciculated with the neurite, and sometimes continued to grow along it for a considerable distance. In other cases, the growth cone turned after its filopodia contacted the neurite and grew roughly parallel to the neurite, maintaining filopodial contact but remaining separate from the neurite. Finally, in some cases, the growth cone retracted from the neurite, although this response was the least common one. Sometimes more than one type of behavior was exhibited in a given encounter, for example, fasciculation was followed by crossing. These experiments show that even in encounters between neurons of the same origin, there is a heterogeneity of responses. One possibility is that this variation is a consequence of the heterogeneous nature of neurons within the dorsal root ganglia. (Supported by NS26386 to MGH)

410.19

AXON INITIATION BY DORSAL ROOT GANGLION NEURONS *IN VITRO*. C.L. Smith and E.M. Munro*, Laboratory of Neural Control, NINDS, NIH, Bethesda, MD 20892.

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

410.20

PURIFICATION AND CULTURE OF IDENTIFIED RAT MOTONEURONS. J.P. Ternaux, J.P. Guéritaud, P. Portalier and N. Sevfritz (SPON: S. Tyc-Dumont). INSERM U6, CNRS URA 142, 280 Bd Ste-Marguerite, 13009 Marseille, France.

It is well known that motoneurons can be retrogradely labelled by various substances. For example, motoneurons of the nucleus hypoglossi and of the oculomotor nuclei are labelled in adult rat, 48 hours following an injection of Rhodamine fluorescent latex microspheres (LUMA FLUOR INC.) in the tongue and the extraocular muscles, respectively. This retrograde neuronal marker as well as immunohistological detection of choline acetyl transferase (ChAT) can be used to detect the presence of motoneurons in histological sections. Following enzymatic dissociation (free calcium and magnesium medium, in presence of trypsin) of the area containing motoneurons, retrograde labelled motoneurones were identified in the neuronal dissociated population. These labelled cells are comparable in size to those detected using ChAT antibody on fixed or living cells. Dissociated neurons had lost their dendritic processes but were able to express new neurites when they were plated for seven to nine days on polyornithine coated petri dishes in DMEM medium. Experiments are actually performed in order to sort labelled motoneurons using various methods, including flow cytometry, and to study the growth of new processes when they are plated on various guidance surfaces.

EPILEPSY: BASIC MECHANISMS III

411.1

NEUROTRANSMISSION AT NMDA RECEPTOR AND HIGH PRESSURE NEUROLOGICAL SYNDROME IN RATS. B.S. Meldrum, M.H. Millan, B. Wardley-Smith* and M.J. Halsey*. Dept. of Neurology, Inst. of Psychiatry, London, SE5 8AF, UK. and *Northwick Park Hospital, CRC, Harrow, Middlesex, UK.

Involvement of excitatory transmission at NMDA receptor within the substantia nigra pars reticulata (SNR) entopeduncular nucleus (EP) and ventrolateral nucleus of thalamus (VLTh) in the high pressure neurological syndrome (HPNS) (tremor myoclonus convulsion) was studied in rats. Focal injection of NMDA (1-10 nmol) into each structure resulted in lowering the threshold pressure for the initiation of tremor and convulsions in hyperbaric conditions. Injection of APH (1-10 nmol) produced the opposite effect i.e. increased threshold pressure for the initiation of tremor and convulsions. The best protection against convulsions (38% threshold increase) was provided by APH injections into the SNR. The same injection into the EP was best in protection against tremor (48%). VLTh injections produced less pronounced effects but these were statistically highly significant.

411.2

CORTICAL BLOOD FLOW AND 0_2 DURING REPEATED HYPERTENSIVE EPISODES AND SEIZURES. J.C. Magee* and N.R. Kreisman. Dept. of Physiology, Tulane Univ. Sch. Med., New Orleans, LA 70112.

Although cortical blood flow (CBF) and oxygenation increase early in status epilepticus, the increase in CBF can drop below the critical level for adequate delivery of 0_2 after an hour or more of seizures. We tested the hypothesis that repeated seizure-associated increases in arterial blood pressure (BP) compromise cardiovascular function, leading to attenuation of CBF and 0_2 delivery. Rats were anesthetized with pentobarbital, paralyzed with curare and ventilated mechanically. Continuous measurements were made of EEG, BP, and the oxidation/reduction state of cytochromes a + a_3. CBF was measured by H_2 washout. Increases in BP from 120 $\pm$ 3 to 198 $\pm$ 2 mm Hg were induced at 5 min intervals by i.v. injection of a mixture of norepinephrine and angiotensin II. CBF increased from 1.1 $\pm$ 0.1 to 3.8 $\pm$ 0.2 $ml.min^{-1}.g^{-1}$ during the increases in BP. No attenuation of increases in BP or CBF were observed over a 2h period. Subsequently, 3-4 generalized seizures were induced individually at 5 min intervals by i.v. injection of pentylenetetrazol. BP increased to 187 $\pm$ 3 mm Hg and CBF increased to 5.4 $\pm$ 0.5 $ml.min^{-1}.g^{-1}$. Cyt a + a_3 oxidized slightly with each BP increase but oxidized more during seizures. The increases in CBF and cortical 0_2 did not attenuate during seizures. Therefore, repeated increases in BP are not sufficient to produce cardiovascular changes resulting in attenuation of CBF and 0_2 delivery during serial seizures. (Supported by the American Heart Association--LA, Inc.)

411.3

THE PATTERN OF 72KD HEAT SHOCK PROTEIN-LIKE IMMUNOREACTIVITY IN THE RAT BRAIN FOLLOWING GENERALIZED STATUS EPILEPTICUS.
D. Lowenstein*, M. Gonzalez*, R. Simon and F. Sharp (SPON: T Koch). Dept. of Neurology, U. of California - SF, San Francisco, CA 94143.

The inducible 72kD heat shock protein (HSP72) is a highly conserved stress protein that is expressed in CNS cells and may play a role in protection from neural injury. We used a monoclonal antibody to HSP72 and immunocytochemistry to localize HSP72 in the rat brain 24 hours following either 30 or 60 minutes of flurothyl-induced status epilepticus. Sprague-Dawley rats were anesthesized with halothane, paralyzed, and ventilated, and remained normothermic, normotensive, and well-oxygenated for the duration of the seizures. Seizure activity was quantified via analysis of the scalp EEG morphology. HSP72-like immunoreactivity (HSP72-LI) appeared in specific brain regions in a graded fashion that correlated with the duration and degree of seizure activity, and the amount of systemic acidosis. Milder seizures produced HSP72-LI limited to layers 2 and 3 of parietal cortex, dentate hilus cells, and CA1 pyramidal neurons. More extensive seizures led to HSP72-LI in layers 2,3, and 5 of parietal, temporal, and pyriform cortex, dentate hilus cells, CA1 and CA3 pyramidal neurons, and certain thalamic nuclei (including VPM, VL, VM, medial and lateral dorsal). These are similar to many (but not all) of the regions of irreversible injury described by Nevander et al (Ann Neurol 18:281-290, 1985) using a similar model. No HSP72-LI was observed in sham-treated controls or flurothyl-treated animals whose seizures were controlled with pentobarbital. Seizure-induced HSP72-LI thus appears to localize to certain regions of seizure-induced injury, and may provide a sensitive method of positively detecting neuronal injury relatively soon after status epilepticus. Whether or not HSP72 synthesis plays a protective or harmful role in the pathogenesis of seizures, or is only a marker for cell injury, remains to be determined.

411.4

ANATOMIC ORIGINATION AND SPREAD OF DISCHARGES IN THE LITHIUM-PILOCARPINE SEIZURE MODEL. A. Handforth and D.M. Treiman* (Spon: J. Engel). VAMC Wadsworth and Dept. of Neurology, UCLA, Los Angeles, CA 90024

We studied the stages of entry into status epilepticus in the lithium-pilocarpine model with the ^{14}C-2-deoxyglucose (2DG) method with the aim of determining the anatomic sites of seizure origination and subsequent spread. Status was induced in 16 LiCl-pretreated rats with pilocarpine, 20 mg/kg IP. 2DG, 40 uCi IV, was given either 1) at the beginning of discrete seizures (D), 2) during waxing-and-waning seizures (WW), 3) during continuous convulsive seizures with either fast-and-slow waves on EEG (FS); or 4) fast continuous spiking (FC). 10 min later the brain was removed; cut sections were apposed to X-ray film. The resulting autoradiographs revealed that the most restricted patterns of seizure-induced hypermetabolism occurred in D and WW subjects with minimal convulsive behavior. Intense activation was limited to orbital cortex and adjoining olfactory areas, substantia nigra, and part of striatum and pallidum. Convulsive activity in D and WW subjects was associated with activation of limbic, rostral neocortical and thalamic areas. In FS, forebrain activation was global but submaximal in caudal neocortex and parts of striatum. In FC, forebrain recruitment was completed. These results suggest that in this model, seizure activity originates in orbital cortex and nearby olfactory areas, then recruits successive anatomic shells.

411.5

NON-CONVULSIVE STATUS EPILEPTICUS PRODUCED BY ETHYLKETOCYCLAZOCINE AND PILOCARPINE CO-ADMINISTRATION. J.S. Kiefer* and G.G. Buterbaugh. Department of Pharmacology and Toxicology, University of Maryland School of Pharmacy, Baltimore, MD 21201.

Since opioid peptides are proposed to play a role in the regulation of seizure threshold and postictal activity, we evaluated the effects of the kappa receptor agonist ethylketocyclazocine (EKC) on pilocarpine induced seizures in rats. Pilocarpine (100 - 250 mg/kg; ip) produced stereotypies, but not convulsions. EKC (4 - 8 mg/kg; ip) caused EEG bursting and sedation but not convulsions. Administration of EKC (4 mg/kg) 5 minutes prior to pilocarpine (100 - 150 mg/kg) consistently resulted in the onset of status epilepticus (SE) in female and male rats within 20 - 25 min. EKC/pilocarpine induced SE was relatively mild compared to the high dose pilocarpine (400 mg/kg) model. SE was nonconvulsive throughout the 3 hour observation period; some rats showed clonic convulsions after about 2.5 hrs. Histological examination revealed mild pathology in the amygdala, piriform cortex, CA1 and CA3 of rats after three hours of SE. Preliminary results suggest age-related differences in sensitivity to the SE produced by EKC/pilocarpine co-administration. These results provide further evidence for a role for opioid peptides in seizure mechanisms in general and in particular, status epilepticus. (Supported in part by PHS NS 20670

411.6

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

Recent research has implicated the midbrain and pontine reticular formation in the propagation of generalized tonic seizures. Specifically, inhibition of the tonic components of generalized seizures [e.g. maximal electroshock (MES), pentylenetetrazol & audiogenic)] has been observed following lesions of the nucleus reticularis pontis oralis (RPO) which include the superior cerebellar peduncles (Browning et al., Epilepsia 22:583, 1981). Furthermore, considerable evidence indicates that norepinephrine (NE) inhibits the tonic components of MES-induced seizures. Therefore, we investigated the effects of microinfusing noradrenergic agonists [NE, phenylephrine (PE) and isoproterenol (ISO)] into the RPO on tonic hindlimb extension (HLE) in the MES test.

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

These data suggest that neither alpha$_1$ nor beta adrenoceptor agonists are effective in attenuating MES-induced seizures when directly applied to the RPO. Additional doses of ISO as well as an alpha$_2$ agonist are currently being examined.

411.7

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

The present study investigated the role of hippocampal dentate granule cells on convulsions and wet dog shakes (WDS) induced by PLO17, a mu opioid receptor agonist. PLO17 (5 ug) was injected into the left ventral hippocampus of rats 14 days after unilateral or bilateral colchicine (COL; 2 ug/site) lesions of ventral hippocampal dentate granule cells. PLO17 injected into control (ACSF-treated) animals produced convulsions and numerous WDS that lasted for less than 1 h. PLO17-induced WDS were significantly reduced in unilateral COL-pretreated rats, and completely inhibited in bilateral COL-pretreated animals. In contrast, generalized motor seizures evoked by PLO17 were potentiated and prolonged in COL-pretreated animals since status epilepticus was observed in both unilateral and bilateral COL-pretreated rats but not in control animals. Furthermore, PLO17 induced widespread, seizure-related damages of CA3/CA1 pyramidal cells in COL-pretreated rats, but not in control animals. These results suggest that dentate granule cells in the ventral hippocampus are essential for the elaboration of WDS. However, these neurons may play an inhibitory role in the spread of seizure activity within the hippocampus or limbic structures.

411.8

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

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

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

411.9

SYNCHRONIZATION OF SEIZURE ACTIVITY IN THE HIPPOCAMPUS AND ENTORHINAL CORTEX OF THE PERFUSED WHOLE GUINEA-PIG BRAIN M. Mühlethaler* and B.A. MacVicar+ (Sponsor: R. Auer). *Department of Physiology, CMU, Geneva, Switzerland and +Neuroscience Research Group, University of Calgary, Calgary, Alberta, Canada.

Intracellular recordings were obtained from the entorhinal cortex (EC) and extracellular recordings were obtained simultaneously from the hippocampus (HIP) in perfused whole guinea-pig brains. Extracellular stimulation of the EC evoked a synaptic potential in EC neurons (and sometimes an antidromic potential) and evoked a population EPSP-spike in the HIP which facilitated at 1Hz. Higher frequency stimulation evoked ictal-type seizure discharges. Intracellular recordings in EC neurons indicated that ictal discharges were composed of a train of paroxysmal depolarizing shifts (PDS) superimposed upon a slow depolarization. EC PDS's were synchronized with population bursts in the HIP. As ictal discharges slowed and then blocked, PDS's occurred independently in the EC and HIP and extracellular stimulation in EC could not evoke synaptic potentials in the HIP. Therefore reciprocal excitatory connections between the HIP and EC could be important in synchronizing ictal discharges.

411.10

DIFFERENTIAL EFFECTS OF COLCHICINE LESIONS OF DENTATE GRANULE CELLS ON WET DOG SHAKES AND SEIZURES ELICITED BY PERFORANT PATH STIMULATION. M.I. Barnes* and C.L. Mitchell, (SPON: E.G. Drust). LMIN, NIEHS/NIH, Research Triangle Park, NC 27709.

Destruction of dentate granule cells (DGC) abolishes wet dog shakes (WDS) elicited by kainic acid (Grimes et al., J. Neurosci. 8:256, 1988) and induced by kindling of the entorhinal cortex (Frush and McNamara, Exp. Neurol. 92:102, 1986). However, the effect of destruction of DGC on seizure activity is not clear (cf. Grimes et al., ibid vs Okozaki and Nadler, Neurosci. 26:763, 1988). Previous studies destroyed DGC in both the dorsal (D) and ventral (V) hippocampus. We compared the effects of destruction of D vs V-DGC on WDS and seizures (S). Destruction of V-DGC almost completely abolished WDS ($\bar{x}$ = 1 ± 3.5) compared to artificial cerebrospinal fluid (ACSF) animals ($\bar{x}$ = 29 ± 3.5). However, both V and D colchicine lesions lowered S thresholds. S threshold was defined as the current required to elicit rearing accompanied by fore-limb clonus. The effect on S threshold was especially pronounced 8 weeks post colchicine lesions of the V-DGC where 7 of 10 animals exhibited seizures at 1 mA or less. Examination at even longer time intervals is thus warranted.

411.11

LONG-TERM ELECTROPHYSIOLOGIC AND BIOCHEMICAL MONITORING IN THE RAT INTRA-AMYGDALOID TETANUS TOXIN CHRONIC MODEL OF EPILEPSY.

D.S. Garant*, Y.-M. Chen*, T.P. Bleck, and R.G. Fariello.
Depts. Neurol. Sci. and Int. Med., Rush Univ., Chicago, IL 60612.

An infusion of picogram amounts of tetanus toxin into rat amygdala results in spontaneous seizures peaking in severity and frequency at 5-10 days. We used awake, behaving male Fisher rats with chronic electrode implants for EEG, and guide cannulae for toxin infusions and in vivo microdialysis.

Confirming our cat studies, rats showed early multifocality in epileptiform EEG activity, with spikes and seizures arising independently from amygdala, hippocampus, and cortex; by the peak of response the infused amygdala was the driving seizure focus, with secondary icti observed consistently from hippocampus.

Focal microdialysis demonstrated decreased basal and veratrine stimulated GABA levels, most consistent at the seizure peak, but appearing to persist some weeks thereafter. Basal levels of Asp and Glu were elevated during the first post-toxin days.

Studies are now underway to examine the effects of this model on hippocampal transmitter levels and synaptic plasticity, and on amino acid receptor binding.

411.12

INTRACEREBRAL MPP+ PRODUCES EPILEPTIFORM ACTIVITY INDEPENDENT OF CATECHOLAMINE RELEASE.

R.G. Fariello, U. Bonuccelli*, and D.S. Garant*.
Dept. Neurol. Sci., Rush Univ., Chicago, IL 60612.

Systemic administration of MPTP produces acute convulsions in all awake species studied. Mice with implanted hippocampal electrodes show EEG spikes and seizures after i.p. MPTP; this activity is dose dependent, attenuated by MAOIs, and exacerbated by diethyldithiocarbamate, suggesting dependence on intracerebral MPP+.

Rats with implanted electrodes and guide cannulae were infused with MPTP or MPP+ into brain. Both compounds were epileptogenic when infused into amygdala or hippocampus; MPP+ appeared faster-acting and more potent. Both amygdaloid and hippocampal infusion of MPP+ resulted in extrafocal discharges at lower doses (150 pmol to 60 nmol); doses of 3-6 umol MPP+ in hippocampus produced focal and behavioral seizures. Hippocampal MPP+ responses were unaffected by reserpinization (5 mg/kg for 3 previous days). No such abnormalities were observed after infusions (30 nmol) into substantia nigra, striatum, or deep prepyriform cortex.

These results indicate a unique excitatory action of MPP+ in brain independent of its acute catecholamine releasing effect.

411.13

EMBRYONIC BEHAVIOR AS A MODEL FOR POSTNATAL EPILEPTIFORM ACTIVITY. R. R. Provine. Dept. Psychology, UMBC, Baltimore, MD 21228.

Chick embryonic behavior is produced by spontaneous bursts of spinal cord neurons first described by Provine and associates (Brain Res. 29 (1971) p. 155; 41 (1972) p. 375; 45 (1972) p. 127; J. Neurobiol. 8 (1977) p. 217). The bursts are synchronized with movement in motile embryos and motor nerve discharges in curarized preparations. The bursts usually begin with a high amplitude "initiating" discharge that is usually followed by a longer, lower amplitude, and more variable "afterdischarge." Before 7 days, bursts consist only of "initiating" discharges. Dual electrode studies indicate that bursts occur almost simultaneously along the rostrocaudal axis of the cord; bursts are initiated at varying cord loci and are then propagated throughout the remainder of the cord. The burstiness and transregional coupling of discharges are not typical of the postnatal cord.

The transregionally synchronized bursts of cord and perhaps other parts of the healthy embryonic CNS may provide a useful model of adult epileptiform activity that does not require the poisons, trauma, or kindling necessary in other model systems. Indeed, some epileptiform disorders may be a postnatal remnant or recapitulation of an embryo-like state caused by developmental disorder, trauma or disease.

411.14

γ-HYDROXYBUTYRATE (GHB) MODEL OF ABSENCE: CORRELATION OF γ-BUTYROLACTONE (GBL) AND GHB LEVELS IN BRAIN WITH EEG CHANGES. O. C. Snead, Dept. Peds., Univ. of AL, Bham, AL 35294 and Childrens Hospital of Los Angeles, Los Angeles, CA 90027.

γ-Hydroxybutyrate (GHB) is a naturally occurring compound which produces absence like seizures when given to animals. The prodrug of GHB, GBL, is used to produce this animal model of generalized absence seizures. A time course study of levels of GBL and GHB in brain in freely moving animals given varying doses of either GBL or GHB was performed under continuous EEG monitoring. The threshold concentration of GHB in brain for absence-like seizure was 20 µg/g. This concentration was achieved earlier and at lower doses with GBL than GHB. GBL was detected in brain in levels one half that of GHB within one minute of GBL administration but dropped off rapidly such that 10 minutes after a dose of GBL the ratio of GHB to GBL was 40. The rapidity and consistency of onset of seizure produced by GBL can be attributed to pharmacokinetic differences between GBL and GHB, since the EEG changes in both GHB and GBL treated animals were dependent on achieving a certain level of GHB irrespective of the concentration of GBL.

411.15

INTRACELLULAR RECORDING OF THE SPONTANEOUS ACTIVITY IN DORSAL HORN INTERNEURONS INDUCED BY ELEVATED CA^{2+} IN MOUSE SPINAL CORDS *IN VITRO*.

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Elevated $[Ca^{2+}]_o$ as well as low $[Mg^{2+}]_o$ induce irregularly recurring "interictal" type spontaneous discharges in dorsal and ventral roots (DRs and VRs) and dorsal horn (DH) gray matter of isolated mouse spinal cords (Czéh & Somjen, Brain Res., in press). In the present study, hemisected spinal cords with attached roots were prepared from 9-14 day-old mice and maintained in an interface chamber at 34.5 C. Suction electrodes were used to stimulate and to record from DR and VR. DH neurons were sought by intracellular micropipette electrodes filled with 4M K-acetate. When $[Ca^{2+}]$ in the bath was elevated to 2.4 or 3.6 mM the membrane potential was about 2 mV more negative than in control solution (1.2 mM) measured in the same 9 cells. The threshold of spike generation did not change. EPSPs evoked by DR stimulation became more prolonged in high $[Ca^{2+}]_o$. In 2.4 or 3.6 mM $[Ca^{2+}]_o$ 17 out of 25 cells were spontaneously active, compared to 7 out of 17 cells in 1.2 mM $[Ca^{2+}]_o$ Spontaneous synaptic activity of 6 cells was synchronous with the dorsal root discharges in elevated $[Ca^{2+}]_o$. We conclude that only a part of the DH cell population is recruited into synchronized spontaneous activity in elevated $[Ca^{2+}]_o$. Waves of depolarization of primary afferent terminals appear to be responsible for the synchronization. (Supported by grants NS17771, NS06233)

411.16

HYPERAMMONEMIA PRODUCES SEIZURES BY NEURONAL DEPOLARIZATION.

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Hyperammonemia can cause seizures. These seizures are thought to be of spinal origin, and to be initiated by the inactivation of Cl^--extrusion from neurons and decrease of postsynaptic inhibition. However, intracellular recordings from spinal motoneurons during the inactivation of Cl^--extrusion by NH_4^+ showed no paroxysmal discharges.

To investigate the occurrence of paroxysmal discharges from spinal motoneurons, monosynaptic excitatory potentials were recorded from ventral roots (VR-EPSP) in barbiturate anesthetized cats with spinal section at L_2. In addition, an extracellular electrode in the ventral horn recorded the field potential in response to stimulation of the ventral roots (ventral root field potential - VFRP). Ammonium acetate i.v., 6 mM/kg bodyweight, decreased the VR-EPSP and, nevertheless, inititated motoneuron discharges. Simultaneously, spontaneous paroxysmal discharges were observed, and the negative component of the VRFP was significantly increased.

These observations indicate that severe hyperammonemia depolarizes motoneurons and inititates spontaneous paroxysmal discharges. Since NH_4^+ inactivates Cl^--extrusion without (and before) depolarizing motoneurons, it is inferred that neuronal depolarization, and not a decrease of postsynaptic inhibition, initiates the seizures due to hyperammonemia.

411.17

GABA-SENSITIVE NIGRAL EFFERENTS MAPPED WITH 14C DEOXYGLUCOSE AUTORADIOGRAPHY IN RAT PUPS.
E.F. Sperber, L.L. Brown, D.M. Smith* & S.L. Moshé. Depts of Neurology & Neuroscience, Albert Einstein College of Medicine, Bronx, NY.

The substantia nigra plays a role in the modification of seizures. In rat pups, nigral infusions of baclofen, a $GABA_B$ agonist, have an anticonvulsant effect. To identify the brain regions involved in the baclofen induced, nigral-mediated seizure suppression, we used 14C deoxyglucose (DG) autoradiography. A unilateral cannula was implanted in the substantia nigra of each of 9 rat pups (age 14 days). Two days later, they were infused with 100 ng/0.25 µl of baclofen, the anticonvulsant dose, or an equivalent volume of saline. Thirty minutes later, the pups were injected with 10 µCi of DG, ip. Analysis of the autoradiograms demonstrated ipsilateral decreases in glucose utilization in the pontine reticular formation, auditory cortex, posterior thalamic nuclei, dorsal striatum and frontal cortex of the baclofen-treated pups as compared with controls. These results suggest that the baclofen mediated suppression of seizures in pups involves the reticular formation and thalamic areas which can have widespread cortical influence.

411.18

CURRENT SOURCE DENSITY ANALYSIS OF HIPPOCAMPAL SEIZURE ACTIVITY IN THE ANAESTHETIZED RAT.
W.J.Wadman, A.J.A.Juta* and G.G.Somjen. Dept. of Exp. Zoology, Univ. of Amsterdam, Amsterdam 1098SM, The Netherlands.

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

411.19

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

The importance of piriform cortex (PC) in experimental models of epilepsy has been demonstrated by the discovery that injection of picomole amounts of convulsants in deep ant. PC evokes generalized seizures (Piredda & Gale, Nature 317:623) and that interictal spikes in kindled animals (Racine et al, Br. Res. 454:251) usually are initiated in PC. CSD analysis was used to investigate the role of association fibers in the initiation and propagation of epileptiform activity in PC. Picrotoxin (Ptx) was applied focally to the ant. PC of urethane anesthetized rats. CSD analysis was performed on time-locked epileptiform activity evoked by shocks to the lateral olfactory tract (LOT). Interictal spikes occurred 5-10mm caudal to Ptx application sites in ant. PC prior to development within the sites. CSD analysis at caudal sites revealed an abnormally large inward current (sink) in sup. layer Ib where association fibers from the ant. PC terminate throughout the PC and entorhinal cortex. This peak was followed immediately by synchronous pyramidal cell firing and a subsequent high amplitude sink in layer Ib. An intriguing finding was that the latency between successive current peaks of epileptiform events was 20ms or integral multiples of 20ms at both ant. and post. sites.

CSD analysis of oscillatory responses to low strength shocks, resembling those recorded in response to odors, has revealed a stereotyped sequence of events that recurs during each cycle of a 20ms oscillation (Ketchum & Haberly, Soc. Neur. Abs. 14:1188). The correspondence in latency of peak dendritic currents during epileptiform activity to the period of synaptic events during this oscillation suggests a possible link between the evolution of epileptiform activity in the PC and the dynamics of the normal response to odor stimulation. Supported by NINCDS grant NS19865 to LBH and NRSA award NS08328 to KLK.

411.20

CROSS CORRELATION VARIABLE LATENCY AVERAGING OF SYNCHRONIZED AFTERDISCHARGES RECORDED FROM HIPPOCAMPAL SLICES.
E.J. Smith*, D.K. Terry*, G.L. Klotz*, R.J. Brady and J.W. Swann. Wadsworth Ctr. for Labs & Res., NYS Dept. of Health, Albany, NY 12201.

Electrographic seizures are recognized to be physiologically complex. One feature of seizure discharges is that the latencies of the synchronized discharges of the afterdischarge are different from one seizure-like event to the next. Thus conventional signal averaging of these events is impossible. In order to begin a detailed analysis of these discharges we have developed an adaptive variable latency averaging procedure. Initially we analyzed after discharges of 2 sec. duration, each consisting of an initial epileptiform burst and 5-15 synchronized discharges. The computer program first detects each individual synchronized discharge in each electrographic event. This is accomplished either by zero crossings in a differentiated signal or by polynomial fitting procedures. After detection, cross correlation is used to align the signal in time with a template. The process is repeated with each succeeding after discharge and each new average updates the template. These procedures result in a smoothly filtered signal for each of the synchronized discharges. Supported by grants NS-18309 to JWS and NS-23071 to RJB from DCDND-NIH.

NEUROMUSCULAR DISEASE

412.1

ULTRASTRUCTURAL PATHOLOGY OF SKELETAL MUSCLE IN PARANEOPLASTIC SYNDROME.
A. Márquez*, Y. Blanco*, P. Tonino*, H.J. Finol, J. Prieto* and L.A. Sosa*. Medicine and Sciences Faculties, Universidad Central de Venezuela and "José Ignacio Baldó" Hospital. Apartado 50587, Sabana Grande, Caracas 1050, Venezuela.

Patients suffering from cancer without muscle metastases may present muscular weakness and wasting. In order to study the ultrastructural basis of these phenomenons muscle biopsies were taken from rectus abdominis of patients with gastric (n=4) and colonic (n=4) cancers and from quadriceps femoris of patients with bronchogenic cancer (n=8). Two different types of damage were observed, biopsies from patients with gastric and colonic cancers presented slightly atrophied fibers mixed with many fibers with segmental necrosis, capillary alterations which included necrosis, and a mononuclear cell infiltration formed by lymphocytes and macrophages; biopsies from patients with bronchogenic cancer exhibited a varied degree of atrophy from slight to severe and occasionally necrosis was seen. Capillary alterations were similar. The differences between these groups could be partially explained because the biopsies were obtained from muscles with different dynamic properties and fiber composition. This work demonstrates that muscle alterations in paraneoplastic syndrome are associated with abnormalities of morphological integrity. Supported by grants from CDCH of UCV (C-03-17-86), Fundación Polar and British Council.

412.2

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

Our objective was to determine whether the gene expression of acetylcholine receptor (AChR) protein is altered in muscles from an animal model of chronic alcoholism. Test rats received a nutritionally complete liquid diet containing 6.7% ethanol. Age and weight-matched control rats were pair-fed an isocaloric diet. After a 16 week diet period, soleus muscles were obtained, and total RNA and poly(A)+ RNA were isolated. GeneScreen Plus membranes were slot-blotted with several levels of each RNA sample, and hybridized with a 32P-labeled complementary riboprobe to detect AChR α-subunit mRNA levels. This riboprobe was prepared by SP6 RNA polymerase transcription of a cDNA clone (BMA 407-12 obtained from J. Boulter) that encodes the AChR α-subunit. Autoradiographs of the membranes were quantitated by laser densitometry. As an internal control, stripped membranes were reprobed with a 32P-labeled riboprobe prepared from a linearized H-ras DNA template. The AChR α-subunit mRNA levels were significantly decreased by 39.6% in solei from the ethanol-fed rats versus pair-fed controls. As expected, H-ras expression was not changed. The reduced synthesis of AChR protein may contribute to the muscle weakness and alcoholic myopathy that commonly occurs during chronic alcoholism.

412.3

GUANETHIDINE NEUROPATHY IN RATS: A MODEL OF HUMAN SYMPATHETIC NEUROPATHY. E.E. Benarroch, J.D. Schmelzer*, K.K. Ward*, P.A. Low. Neurophysiology Lab., Mayo Clinic, Rochester, MN 55905

Guanethidine (GU) produces chemical sympathectomy in rats (GUSX). We examined GUSX as a model of human sympathetic neuropathy. Rats (250-350 g) received GU (40 mg/k i.p.) or saline for 6-12 w. Subsequently, they were anesthetized (Inactin) and canulated for mean arterial pressure (MAP) and heart rate (HR) recording. Tyramine (Tyr, 30 mmols) and phenylephrine (Phen, 0.06 or 0.6 μM) were infused intravenously. Norepinephrine (NE) levels in plasma, femoral artery (FA), superior cervical ganglion (SCG), atrium, and sciatic and vagus nerves were measured by HPLC. As compared to controls, GUSX rats showed (a) lower basal MAP but not plasma NE, (b) blunted responses to Tyr in terms of increase in MAP (3.5±6.8 vs. 90±3.3 mm Hg; $p<0.0001$) and plasma NE (0.12±0.11 vs. 6.16±0.41 ng/ml; $p<0.0002$), (c) exaggerated pressor responses to Phe (e.g., with 0.06 μmol, 59.2±3.2 vs. 12.3±6.74 mmHg, $p<0.001$), and (d) lower NE levels (ng/g) in all tissues (e.g., SCG: 0.54±0.14 vs. 16.73±3.37, $p<0.003$; FA: 0.09±0.02 vs. 1.62±0.15, $p<0.001$). There was correlation between NE content in SCG and FA and responses to Tyr and Phen, respectively. There was no correlation between NE levels in plasma and tissue. Thus, (a) GUSX reproduces findings of human sympathetic neuropathy and (b) pharmacological responses, but not basal plasma NE, reflect the degree of NE depletion.

412.4

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

We have previously reported the presence of endoneurial hypoxia, ischemia, impairment of the blood-nerve barrier and reduction of norepinephrine (NE) and 6-keto-prostaglandin $F_{1\alpha}$ in chronic streptozotocin diabetic neuropathy (SDN) and interpreted these findings as suggesting the involvement of OFR.[1,2] We now report our studies on sciatic nerve conjugated dienes, hydroperoxides, NE, and malondialdehyde (MDA) in SDN at 1, 4, and 12 months in male SD rats. Severe hyperglycemia was present throughout in SDN. Conjugated dienes were consistently increased at all time points, hydroperoxides were consistently reduced, and MDA was not significantly different in SDN when compared with controls.

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

1. Tuck RR, Schmelzer JD, Low PA. Brain 107:935-950, 1984.
2. Ward KK, Low PA, Schmelzer JD, Zochodne DW. Brain 112:197-208, 1989.

412.5

PATCH CLAMP MEASUREMENTS OF VISCOELASTIC PROPERTIES AND MECHANOELECTRIC TRANSDUCTION IN DYSTROPHIC MUSCLE MEMBRANE. B.J. Cooper* and O.P. Hamill. Depts. Pathology and Neurobiology and Behavior, Cornell Univ., Ithaca, NY 14853.

Recently, the gene which is defective in Duchenne muscular dystrophy (DMD) has been identified and its product, a 400 kD protein named dystrophin, has been shown to be absent or abnormal in patients with DMD as well as in the canine model of the disease (CXMD). However, at this stage there is no direct evidence on the mechanism by which dystrophin actually maintains healthy muscle function nor why its absence results in a progressive muscular weakness. Based upon its subcellular localization to the cytoplasmic membrane face, its cytoskeletal characteristics, the evidence of membrane injury in DMD, and the rise in intracellular Ca ions, a plausible hypothesis is that dystrophin plays some role in stabilizing the plasma membrane of healthy muscle cells, in its absence, the membrane may be more fragile and leaky to ions such as Ca^{++}. Another possible pathogenic feature is that there is abnormal activation of the stretch activated (SA) Ca^{++}-permeable channel that has been reported in cultured muscle cells. To test these ideas we carried out patch clamp measurements of acutely isolated adult muscle taken from toe muscle biopsies of normal dog as well as CXMD dog. We detected no difference between normal and dystrophic muscle in elasticity, as judged by the pressure required to break the membrane. In acutely dissociated adult muscle we were unable to detect SA channels in either healthy or dystrophic muscles, which may either reflect a real absence in fully developed muscle, or a particularly low channel density. Supported by the Cornell Biotechnology Program.

412.6

IMPAIRED SKIN FIBROBLAST CARNITINE UPTAKE IN HETEROZYGOTE PARENTS OF CHILDHOOD CARNITINE-RESPONSIVE CARDIOMYOPATHY. I. Tein*, D.C. De Vivo, S. Servidei*, E. Bertini*, F. Bierman*, S. DiMauro. Columbia-Presbyterian Medical Center, New York, NY 10032.

Evidence is emerging that primary systemic carnitine deficiency, a potentially lethal but treatable inborn error of fatty acid oxidation, involves a cellular defect in carnitine uptake (Eriksson et al., Eur J Pediatr, 147: 662, 1988; Treem et al., NEJM, 319: 1331, 1988). We also studied carnitine uptake in cultured skin fibroblasts from two unrelated children with carnitine-responsive cardiomyopathy and myopathy and present new studies in the heterozygote parents of one patient. The patients had low muscle and serum carnitine and a severe renal leak and responded dramatically to oral carnitine supplementation.

[^{3}H]L-Carnitine uptake was determined *in vitro* under linear time kinetics. Substrate concentrations were varied from 0.1 to 1,000 μM. Physiological uptake was determined at carnitine concentrations between 0.1 to 50 μM. Nonspecific uptake was determined at a concentration of 10 mM. The two patients showed no significant uptake in the physiological range, implying a marked deficiency in the specific, high affinity, low concentration uptake mechanism. Both presumed heterozygote parents of patient #1 had normal carnitine substrate concentration K_m values but significantly decreased V_{max} values for carnitine uptake (40% of controls). This, in addition to the reduced serum carnitine levels in both parents of patient #2, supports an autosomal recessive inheritance pattern.

412.7

ANTIBODIES FROM PATIENTS WITH LAMBERT-EATON SYNDROME RECOGNIZE BOTH PLASMA MEMBRANE AND CYTOSOLIC ANTIGENS IN CHROMAFFIN CELLS. M.P. Viglione, C.E. Creutz* and Yong I. Kim. Depts. of Neuroscience, Pharmacology, Biomedical Engineering and Neurology. University of Virginia, Health Sciences Center, Charlottesville, VA 22908.

Lambert-Eaton syndrome (LES) is a paraneoplastic (70% have associated small-cell carcinoma of the lung or SCCL) neurological disorder characterized by the decreased release of ACh at the neuromuscular junction. As LES autoantibodies have been shown to inhibit the function of voltage-dependent calcium channels in bovine adrenal chromaffin cells (Science, 239:405, 1988), we were interested in whether LES antibodies recognized calcium channel subunits on Western blots of chromaffin cell fractions. Post-nuclear supernatant, post-microsomal supernatant (cytosol) and plasma membrane (PM) fractions were obtained by sucrose gradient centrifugation, run on reducing SDS PAGE and Western blots probed with 0.1 mg/ml IgG from 9 LES patients, 1 SCCL patient and 6 normal individuals. Seven of the nine LES IgGs interacted with a wide range of PM (45-150 kDa) as well as cytosolic (25-135 kDa) proteins with no apparent pattern in regard to the association of LES with SCCL, efficacy of the LES IgG in reducing I_{Ca} or recognition of calcium channel subunits. Preincubation of LES IgG with cytosol before reacting with Westerns abolished IgG interaction with the PM fraction, confirming the presence of a cytosolic antigen that is also present on the PM. One control IgG reacted with a protein also recognized by LES or SCCL IgG.

Thus, LES autoantibodies react with antigens located in the PM and cytosol. It is not known whether this reaction is mediated by a population of autoantibodies distinct from those implicated in the functional inhibition of I_{Ca}. Therefore, we are investigating whether LES IgG is as effective in reducing I_{Ca} after interaction with cytosolic antigens.

412.8

MOLECULAR CLONING OF AN ANTIBODY THAT CROSS REACTS WITH DNA AND MYELIN FROM A PATIENT WITH CHRONIC LYMPHOCYTIC LEUKEMIA AND PERIPHERAL NEUROPATHY. **LA Spatz, KK Wong, M Williams and N Latov. Lab. of Neurology, Columbia University, College of Physicians and Surgeons, New York, NY 10032.**

Clones coding for the variable heavy (V_H) and light (V_L) chain genes of the IgM,k monoclonal autoantibody that binds to DNA and myelin from a patient with chronic lymphocytic leukemia (CLL) and peripheral neuropathy were isolated from a cDNA library, constructed with polyA RNA from a human hybridoma line secreting the autoantibody.

Sequence analysis revealed that the V_H gene has 96% homology to the VH26 germline gene which is a member of the V_HIII gene family and frequently utilized in anti-DNA antibodies. The V_K gene has 99% homology to the V_g gene of the K_KIIIa subgroup of the V_K gene family. Genes of the V_KIIIb subgroup which is assigned a separate subgroup from V_KIIIa on the basis of a single amino acid difference, have frequently been observed in antibodies with rheumatoid factor activity. This supports the theory that autoantibodies are often encoded by a restricted repertoire of variable region genes. Further comparison of the V_H and V_K sequences of the autoantibody with the germline sequences, suggest that the autoantibody arose by somatic mutation in the hypervariable regions which can alter antibody specificity and lead to autoimmune disease.

412.9

CLUES IN THE PATHOGENESIS OF THE MOTOR NEURON DEGENERATION (Mnd) MOUSE. **J.E. Mazurkiewicz*, L. Callahan* and A. Messer (SPON: G.A. Banker). Dept. of Anat., Cell Biol. & Neurobiol., Albany Med. Col. and Sch. of Public Health Sciences, SUNYA, Albany, NY 12208.**

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

(Supported by NIH NS24426 and the ALS Association)

412.10

DIFFERENTIAL EXPRESSION OF FUNCTIONAL ABNORMALITIES IN MITOCHONDRIA FROM CARDIAC AND SKELETAL MUSCLE OF CHF-146 STRAIN DYSTROPHIC HAMSTERS (DH). J.H. Thakar, P.L. Johnson and S.K. Bhattacharya. Univ. of Tenn., Memphis, TN 38163.

Membrane-mediated excessive intracellular Ca accumulation (EICA) is a fundamental pathogenetic event associated with chronic muscle degeneration in Duchenne muscular dystrophy (DMD) (Bhattacharya & Johnson, Proc. 14th World Congress of Neurology, 881, 1989). EICA, necrosis, global muscle weakness, cardiomyopathy with EKG changes, and impaired mitochondrial function have been reported in the BIO 14.6 DH. Because of significant EICA in the CHF-146 strain DH (Soc. Neurosci. Abstr., 14:142, 1988), we studied the function of mitochondria (MIT) from cardiac and skeletal muscles of young (30-day) and old (>365-day) DH. CHF-148 strain normal hamsters served as controls. MIT were isolated and studied according to Thakar et al. (BBA, 314:8, 1973). MIT from skeletal muscles of young DH were uncoupled [ADP/O=0 and RCR=1], whereas those from the older DH were relatively normal. Conversely, myocardial MIT from young DH were functionally normal, although those from old DH revealed significant impairment of oxidative phosphorylation. We conclude that muscle weakness and MIT dysfunction in DH is expressed at an early age in the skeletal muscle, whereas these changes in the ventricular myocardium are manifested later. This parallels the clinical course in DMD, and further justifies the use of CHF-146 strain DH as an animal model for the study of DMD. (Supported by NIH Grant #AR-38540 to SKB.)

412.11

EFFECT OF LAMBERT-EATON MYASTHENIC SYNDROME (LEMS) PLASMA ON $^{45}Ca^{2+}$ UPTAKE IN RAT FOREBRAIN SYNAPTOSOMES. S.J. Simas and W.D. Atchison. Dept. of Pharmacol./Toxicol., Mich. State Univ., E. Lansing, MI 48824

LEMS, a disorder of neuromuscular transmission involving the reduction of nerve-evoked release of acetylcholine, has been transferred passively to mice by chronic injection of immunoglobulin G or plasma from affected humans. An autoantibody to the presynaptic Ca^{2+} channel has been suggested to mediate the pathogenesis of this disease. The objective of this study was to determine whether acute application of plasma from a patient with LEMS reduces depolarization-dependent Ca^{2+} uptake into synaptosomes. Rat forebrain synaptosomes were incubated under pure O_2 for 1 hr with varying concentrations of LEMS or control plasma (% total incubation volume). Heat inactivation of the LEMS plasma was necessary to prevent aggregation of the synaptosomes. Ca^{2+} uptake ($^{45}Ca^{2+}$) was measured during a 10 sec incubation period with either resting (5 mM KCl) or depolarizing (77.5 mM KCl) solution. Net potassium-stimulated influx was inhibited 15-20% by 6 and 12% LEMS plasma, while 24% plasma caused an approximate 30% reduction compared to synaptosomes incubated with equivalent concentrations of control plasma. These results indicate that LEMS plasma blocks depolarization-dependent uptake of Ca^{2+} into central axon terminals. This result is consistent with a site of action associated with presynaptic voltage-dependent Ca^{2+} channels in the nerve terminal. (Supported by the Muscular Dystrophy Association.)

412.12

Quantitative Analysis of Pendular Leg Motion in Normal and Spastic Subjects. D.C. Lin* and W.Z. Rymer (SPON: W.T. Rainey) Northwestern Med. School and Rehab. Inst. of Chicago, Chicago, IL 60611.

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

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

Work supported by NIH grant NS-19331.

412.13

PRESENCE OF DYSTROPHIN-LIKE MOLECULE IN NEURONS OF NORMAL AND DUCHENNE MUSCULAR DYSTROPHY HUMAN FETAL CULTURES. S. Torelli*, F. Muntoni*, A. Clerk*, P.N. Strong* & F. Gremo Dept.of Cytomorph.,Inst.of Child Neuropsych.,School of Med., Cagliari, Italy; Dept.of Paediatr.,Royal Postgrad. Med. School, London, England

Duchenne Muscular Dystrophy (DMD) is the result of the deficiency of a specific protein called dystrophin. Dystrophin is thought to be a cytoskeletal protein, binding to the inner plasma membrane and is present in all the types of muscle cells and in the brain. Since approximatly 30% of DMD patients are considered to be mentally deficient, it can be hypothesized that dystrophin deficiency can cause CNS defects. We have performed immunocytochemical studies on normal and DMD human fetal brain cultures, using polyclonal antibodies against dystrophin. Results showed that in normal cultures only neurons expressed dystrophin, whereas astrocytes and their precursor were always negative. Dystrophin-like staining was mainly present in cell bodies, neuronal processes were only faintly stained. DMD neurons showed peculiar morphological features, like membrane expansions similar to pseudopodia. Dystrophin-like staining was less intense respect to normal and the protein was unevenly distributed in patches in both cell bodies and processes.

413.1

SUBSTRATE-DEPENDENT RESPONSES OF NEURITES TO DC ELECTRIC FIELDS *IN VITRO*. Ann Rajnicek*, John Cork* and Kenneth Robinson* (SPON:C. Jaeger). Dept. of Biol. Sci., Purdue Univ., W. Lafayette, IN 47907.

It is known that neurites from the disaggregated neural tube cells of *Xenopus* embryos show a striking orientation toward the cathode (negative pole) in response to weak DC electric fields applied to cells growing on tissue culture plastic (Falcon). Since the substrate is known to affect neurite initiation and may also play a role in neurite guidance *in situ*, we have extended these studies to include the field-induced responses of *Xenopus* neurites growing on various substrates. Cells were grown in electrical field chambers constructed from tissue culture plastic dishes that were untreated or coated with poly-L-lysine (PL), laminin (L) or PL with an overlying layer of laminin (PL+L). Data were collected as photographs of each cell in the dish at the conclusion of the experiment. Negatives were analyzed with the aid of a digitizer interfaced with a microcomputer. Cells grown on PL and PL+L showed a strong orientation of neurites toward the anode but neurites on L substrates grew toward the cathode. Initiation sites were predominately on the cathodal sides of the cell bodies, regardless of substrate. Control cells showed random neurite initiation as well as random overall growth on all substrates. Experiments are currently underway to investigate the effect of surface charge on field-induced neurite guidance.

413.2

PATTERNED GROWTH OF HIPPOCAMPAL NEURONS ON MICROCIRCUITS. B.C.Wheeler, J.G.Eden* and G.J.Brewer. Southern Illinois Univ., Springfield, IL 62794 and Univ. of Illinois, Urbana, IL 61801.

To study synaptic specificity in CNS neurons, it would be helpful to provide conditions in which a limited number of growing axons and dendrites can be monitored for connective choices. Limited choices for neuronal connections can be controlled by growth of neurons on a patterned substrate (Soc. Neurosci. Abstr. 14:578). We have refined this UV laser-etched poly-lysine substrate by using a quartz mask fabricated with conventional electron beam lithography used in microelectronics. This permitted optimization of path width, path length and node size in a single complex array. Rat hippocampal neurons were dissociated and grown at low density (6,000 cells/cm^2) in defined medium (Brewer & Cotman, Brain Res., in press). A 3 μm path width was important to restrict process outgrowth to a single neurite per path. A node size of 30 μm diameter facilitated control of soma spacing. Apical dendrites typically oriented away from putative input to the proximal dendrites. Because individual cells can be regularly identified *in vitro* by light microscopy over many days, observations of process growth and "choices" will be possible. This model will facilitate studies of trophic influences on synaptogenesis. Supported in part by SIUSM Central Research Committee.

413.3

DIRECTED NEURITE OUTGROWTH FROM ETCHED COLLAGEN FIBERS. E. Wong, A. Rizvi*, D. Christiansen*, H.M. Geller and F. Silver*. Biomaterials Ctr. and Dept. of Pharm., UMDNJ-Robert Wood Johnson Med. Sch., Piscataway, NJ 08854.

Collagen is an important substrate allowing attachment of neurons and their neurites. We previously reported neurite extension on collagenase-etched collagen fibers. In this study, we investigate neurite extension on etched fibers using immunocytochemical techniques.

Cortices were taken from the brains of Sprague-Dawley fetal rats at day 15 of gestation. Cortical cells were cultured for 48 to 96 hours on coverslips containing etched fibers. These cells were then fixed in an acid-alcohol solution and neurofilament-specific monoclonal antibody RT97 was added to the cells on coverslips, followed by the addition of fluorescein-conjugated IgG. The coverslips were mounted onto glass slides in glycerol containing 0.1% p-phenylenediamine. Neurite extension on etched fibers was examined under a microscope equipped with Normarski phase-contrast optics and an epifluorescence illuminator with a fluorescein filter.

Immunofluorescence of neurites and neurite fascicles was detected along the long axes of etched fibers. We conclude that etched collagen fibers allow the attachment of neurites and direct their extension.

Supported by a fellowship from the New Jersey Center for Advanced Biotechnology and Medicine, and a grant from NIH to H.M.G.

413.4

OPIOID PEPTIDES STIMULATE THE GROWTH OF CGRP-IMMUNOREACTIVE DORSAL ROOT GANGLION AXONS IN ORGANOTYPIC TISSUE CULTURE. L. Takes* and S. Jeftinija (SPON: J. Carithers). Dept. Veterinary Anatomy, Iowa State University, Ames, Iowa 50011.

Ganglionic cells in the dorsal root ganglion (DRG) regenerate their axons after axotomy both in vivo and in vitro. Earlier reports have shown that opioid peptides stimulate outgrowth from the DRG in culture (Ilyinsky et al., 1987, Neurosci. 22:719-735.). The aim of the present study was to determine if the stimulatory action of opioids is exerted on neuronal elements directly, or indirectly by stimulating the growth of nonneuronal elements. By using immunocytochemistry we were able to identify calcitonin gene-related peptide (CGRP) positive axons and observe their relationship with the total population of cellular elements. DRG of 11 to 13-day-old rats were dissected and cultivated on chicken plasma-coated slides in Petri dishes or in rotating test tubes. Neurite outgrowth, size of the growth zone, and cell composition were estimated. Opioids stimulated both proliferation of nonneuronal elements and growth of CGRP positive neurites. A strong growth-promoting effect of opioids on neuronal and nonneuronal elements on tissue in culture has been observed in cultures grown in the presence of cytosine arabinoside. Opioids promoted the rate of migration of glial elements manifested as an increase in size of growth zone. The effect on the growth of CGRP positive neurites was dependent on the migration of glial elements. The present observations provide direct evidence that opioids promote the growth of DRG neuron CGRP immunoreactive neurites. In addition, the observations that glial migration outpaced neurite outgrowth, and that CGRP positive neurites almost always terminated in intimate contact with glial elements suggests that the stimulatory effect is an indirect one through the promotion of proliferation and migration of glial elements.

413.5

MORPHOLOGICAL FEATURES AND NEURAL DIFFERENTIATION OF NEUROBLASTOMA (N-2A) INDUCED BY REDUCED MEDIA SERUM CONCENTRATIONS. J. Rossi III, A.A. Messier*, E. Heyder* and A. B. Callahan*. Naval Submarine Medical Research Lab., Biomedical Sciences Dept., Groton, CT 06349.

Previous studies demonstrated that cloned cells extend neurites when incubated in media with low serum concentrations. In these studies N-2A cells were cultivated in media containing 10% fetal bovine serum (FBS). After 24 hrs the cells were subcultivated in media containing either 10%, 5%, or 1% FBS; or media containing 10%, 5%, or 1% NU-SERUM V (NSV). The cells were morphologically characterized after 24, 48, 72 and 96 hrs incubation. Reduced media serum concentration increased the proportion of differentiated cells, increased the neurite yield, increased the length of the neurites, increased the number of varicosities on the neurites, and increased the number of sites where varicosities on neurites from different cells overlapped or abutted. For all measures NSV substitution reliably induced greater neuritogenesis than equal concentrations of FBS.

413.6

THE EXPRESSION OF THE CLASS III BETA-TUBULIN ISOTYPE IN DEVELOPING NEURONS IN CULTURE. A. FERREIRA, A. CACERES, L. REBHUN AND A. FRANKFURTER. (SPON: G. Hall). Dept. of Biology, University of Virginia, Charlottesville, VA 22901

The expression of the class III beta-tubulin isotype was studied in cerebellar macroneurons and hippocampal pyramidal neurons in culture by means of a monoclonal antibody (clone J-1). The results obtained indicate that during cell nerve differentiation there is a progressive increase of this tubulin isotype, a phenomenon which is accompanied by modifications in its intracellular distribution and in its ability to incorporate into microtubules. The time course of its induction is different from the increase in total polymeric tubulin and the induction of MAP-2 and tau which parallel axonal and dendritic growth. On the contrary, its induction profile is similar to that of MAP-1a and MAP-1b. Besides, our results show that the appearance of colchicine-resistant microtubules containing this tubulin isotype is coincident with the one of stable microtubules containing high molecular weight-MAPs.

Taken collectively, the present observations suggest that the class III neuron specific beta-tubulin isotype is not a primary factor involved in the regulation of microtubule assembly during neurite growth, but that it may contribute to determine some unique binding property of MAPs to specific microtubule subsets.

413.7

GANGLIOSIDE ENHANCED NEURITE GROWTH: EVIDENCE FOR A SELECTIVE INDUCTION OF HIGH MOLECULAR WEIGHT MAP-2. A. Caceres*, A. Ferreira*, C. Landa*, and J. Busciglio* (SPON. L. Lampson), Inst. Ferreyra, Casilla de Correo 389, 5000 Cba. Argentina, and Dept. Quimica Biologica (U.N.C.) 5016 Cba. Argentina.

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

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

These observations suggest that MAP-2 induction in neuroblastoma cells may led to a further differentiation of neurites equivalent to that observed in mature neurons. Finally, our results indicate that gangliosides per se are not neuritogenic factors but rather substances capable of greatly enhancing cell derived influences which affect the neurite outgrowth response of neuroblastoma cells and the type of MAP that they express.

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

413.8

TYROSINE KINASES DERIVED FROM NERVE GROWTH CONE GLYCOPROTEINS. N. Cheng*, E. Suwita* and N. Sahyoun. Wellcome Research Laboratories, Research Triangle Park, NC 27709.

Glycoproteins were isolated from neonatal rat brain growth cones by wheat germ agglutinin-affinity chromatography. Phosphorylation of poly glu4tyr revealed the presence of a tyrosine kinase activity (1 nmol/min/mg protein) which required the presence of both $MgCl_2$ and $MnCl_2$. Tyrosine phosphorylation of several endogenous polypeptides was also observed in the presence of $MnCl_2$. Endogenous substrates included tubulin and a Mr 115,000 polypeptide whose electrophoretic mobility could be altered by the presence or absence of reducing agents. Insulin stimulated the phosphorylation of a Mr 90,000 polypeptide while IGF-I elicited phosphorylation of 90,000 and 97,000 polypeptides corresponding to the β-subunit of the insulin and IGF-I receptors, respectively. Tyrosine phosphorylation of endogenous substrates was confirmed by phosphoamino acid analysis, alkali hydrolysis and immunoprecipitation with phosphotyrosine antibodies. In contrast to growth cones, synaptosomal glycoproteins were relatively lacking in protein tyrosine kinases, suggesting that the expression of these enzymes may be regulated by the stage of neuronal development, and that they may play a significant role in growth cone transmembrane signalling.

413.9

Mathematical Analysis of Growth Cone Motility

H. M. Buettner* and R. N. Pittman, Department of Pharmacology, University of Pennsylvania School of Medicine, Philadelphia, PA 19101-6084

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

1) measurement of the root-mean square speed and persistence time of individual growth cones (see: G.A. Dunn, Agents Actions [Suppl] 12 14, 1983)
2) measurement of growth cone shape and orientation based on the moments of growth cone area (see: G.A. Dunn and A.F. Brown, J. Cell Sci. 83 313, 1986)

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

413.10

INTERACTIONS BETWEEN SYMPATHETIC GROWTH CONES IN VITRO. J.K. Ivins and R.N. Pittman. Dept. of Pharmacology, Univ. of Pennsylvania Sch. of Med., Philadelphia, PA 19104.

The nerve growth cone is a highly motile and dynamic structure intimately involved in such diverse phenomenon as neurite outgrowth, pathfinding, target cell recognition and synaptogenesis. Recent evidence has suggested that environmental factors such as contact with other cell surfaces may be important for regulating growth cone motililty. We have used time-lapse videomicroscopy to determine events following contact between growth cones of sympathetic neurons from the rat superior cervical ganglion (SCG) and other cell surfaces, including other sympathetic growth cones and neurites.

45% of the contacts between growth cones of two sympathetic neurons resulted in the collapse of growth cone structure of one, but never both growth cones. Only 12% of the contacts between growth cones and neurites resulted in growth cone collapse. Contact with other cell surfaces, either red blood cells or fibroblasts never resulted in collapse. These studies suggest that the SCG may be composed of two subpopulations of neurons with different cell surface determinants, and that these determinants are more concentrated on the surface of growth cones than on neurites. Approximately half of the neurons of the SCG project rostrally, approximately half project caudally. Experiments are in progress to determine if these represent the two subpopulations that we detect in our culture system. Studies using the calcium indicator dye fura2 are also in progress to examine the mechanism of this collapse phenomenon. (*Supported by the McKnight Foundation and the PMA*).

413.11

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

In a previous report (Kalil and Perdew, *J. Neurosci.*8:4797-4808, 1988), we described a 33kDa membrane protein whose expression declined sharply during development of the hamster CNS. To obtain insight into the growth related function of this protein, we examined its intracellular distribution in growing neurons in culture using fluorescence immunocytochemistry. PC12 cells and dissociated superior cervical ganglion (SCG) cells from 3-day old hamsters were grown in culture on polylysine coated coverslips. Immunocyto-chemistry was carried out on fixed permeabilized cells with monoclonal antibodies against the 33kDa protein. In the presence of NGF, PC12 cells and SCG neurons exhibited extensive process outgrowth with growth cones of various sizes and morphologies. Longer processes had relatively small simple tips whereas shorter neurites ended in large, spread out lamellipodia from which prominent filopodia often extended. Cell bodies, neurites, and growth cones of PC12 cells and SCG neurons were brightly fluorescent. The staining had a diffuse granular appearance, but in large lamellipodia the fluorescence resembled a filamentous mesh-work. Parallel experiments in which rhodamine-phalloidin was used to stain actin filaments and anti-tubulin antibodies were used to stain microtubules suggest that the distribution of the 33kDa protein is not identical with that of either of these cytoskeletal components. Preliminary results also suggest that the 33kDa protein is neuron-specific, since neither fibroblasts nor glial cells in the SCG cultures were immunoreactive. Although neuronal cell bodies were immunofluorescent prior to neurite outgrowth, PC12 cells exhibited significantly brighter fluorescence following NGF stimulation. Taken together, these results suggest that the 33kDa protein may be associated with growth cone function during process outgrowth. (Supported by NIH grant NS14428 to K. Kalil.)

414.1

AN IMPROVED STAINING METHOD FOR SENILE PLAQUES AND NEUROFIBRILLARY TANGLES IN ALZHEIMER'S DISEASE: QUANTITATIVE COMPARISON WITH OTHER TECHNIQUES. R. Guntern*, C. Bouras*, and P.R. Hof (SPON: J.H. Morrison). Dept. of Psychiatry, Univ. of Geneva, Switzerland, and Research Institute of Scripps Clinic, La Jolla, CA, USA.

In an attempt to better quantify the number and distribution of the neuropathological hallmarks of Alzheimer's disease we used a modification of the classical thioflavine S staining method for neurofibrillary tangles (NFT) and senile plaques (SP). This method can be applied to human brains collected after a short post mortem delay and fixed for immunohistochemical purposes, as well as to brains kept in formalin for years or to paraffine-embedded materials.Sections were pretreated with 0.25% $KMnO_4$ and then immersed in 1% $K_2S_2O_4$ and 1% oxalic acid. Paraffin-embedded tissues were then treated with 1% NaOH and 3 ml of 30% H_2O_2. Thioflavine was then diluted up to 0.0125% instead of 1%. This treatment removes lipids autofluorescence. The method allows for the visualization of as much as 50% more SP and NFT as compared to the classical thioflavine S method or to argentic impregnations. Amyloid accumulation in the wall of small vessels was frequently observed. Extravascular amyloid deposits were also evident. A comparable method enhanced peptide immunostaining in human brains kept in formalin for 40 years. Finally, a very accurate automatic quantification of NFT and SP using a computer-assisted image analysis system was made possible by the heightened quality of the staining. This improved technique might be useful to further analyze the regional and laminar distribution of the pathological lesions in senile and presenile cases of Alzheimer's disease.

414.2

ALZ-50 IMMUNOREACTIVITY PRECEDES PHF FORMATION AND NEURITIC CHANGE IN DOWN'S SYNDROME. L.A. Mattiace, D.W. Dickson*, Y.S. Kress* and P. Davies*. Department of Pathology, Albert Einstein College of Medicine, Bx., NY 10461.

By sharing a constellation of neuropathological and clinical changes, Down's Syndrome (DS) appears to be an appropriate model to examine the sequence of changes which develop over the course of Alzheimer's Disease (AD). Twenty-four cases of DS ranging in age from two days to 60 years old were examined. Consecutive vibratome sections from the frontal cortex, hippocampus and the basal forebrain were immunostained with Alz-50, Ab39 (PHF), anti-ubiquitin (UBQ), BetaSP (amyloid) and thioflavin S. Sections immunostained for Alz-50 were also processed for EM.

Consistent Alz-50 staining of neurons was seen in infants up to 2 years of age. Age 29 appeared to be the earliest reexpression of Alz-50 positive neurons coinciding with an extensive array of immunoreactive beaded processes that were absent in the Alz-50 negative cases. Although both beaded processes and neurites appeared to coexist in Down's in the earlier stages of AD changes, these beaded processes disappeared as the AD pathology progressed to extensive neuritic change in the neuropil. Although Alz-50 usually immunostained a greater number of neuropil neurites than either Ab39 or UBQ, in two cases UBQ positive neurites were predominant. Alz-50 cells also were temporally coexistent with a small number of senile plaques and NFT, but were never associated with more than a few neurites in the neuropil. Alz-50 positive cells however did occur without deposition of amyloid in some cases. Thus, reexpression of Alz-50 immunoreactivity may precede both amyloid deposition and the extensive appearance of neurites in the neuropil.

414.3

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

The ADAP was purified ~1250 fold by differential centrifugation, detergent extraction and solubilization from Alzheimer's disease brain tissue homogenate and analyzed by three HPLC methods. The methods were: gel permeation (Beckman Spherogel TSK 4000 PW, in both native and denaturing conditions), Reversed phase by both C-8 (Beckman Ultrasphere Octyl 5u) and C-3 (Beckman Ultrapore RPSC) and finally affinity chromatography with the IgG iso-type of ALZ50, P42 (Beckman Ultraffinity-EP). Activity in column fractions was monitered both by UV (220 and 280 nm) and by ELISA using the ALZ50 antibody. The gel permeation column with native conditions showed a >100 kDalton immunoreactive material and the denatured conditions showed a multicomponent group clustered about molecular weight 64-68 kDaltons. The reverse phase results were: C-8 showed 4 peaks of activity, C-3 showed 2 peaks (the second peak was clearly multi-component). A single ("sharp") peak of immunoreactivity was eluted from the affinity column. Some fractions were analyzed by SDS-PAGE/Western Blots. The affinity column purified material showed triplet bands of activity by this analysis.

It is clear that ADAP is a mix of immunogenically similar proteins that may be iso-forms. Further work to purify and sequence ADAP is presently underway.

414.4

ATP-DEPENDENT LOSS OF ALZ-50 IMMUNOREACTIVITY WITH A68. I.Vincent, and P Davies*. Dept. of Pathology, Albert Einstein College of Medicine, Bronx, NY, 10461.

Preincubation of the Alz-50 immunoreactive proteins (A68) with 5mM ATP at 37^0C results in a subsequent loss of Alz-50 reactivity with A68 on immunoblots. This loss of antigenicity occurs progressively with time, being optimal after a 1 hour incubation. A dependency on temperature is indicated by prevention of the inhibition by boiling of the tissue, or by incubation at 4^0C. Other nucleotide triphosphates do not substitute for ATP, indicating that the dependence on ATP is stringent. The hydrolysis of ATP is also necessary for the inhibition, since the nonhydrolyzable analog Adenosinc-thio-triphosphate is not effective. Similarly, the ATPase inhibitors vanadate and oligomycin block the ATP-induced loss of the Alz-50 epitope. Upon further characterization, it was found that certain protease inhibitors i.e., TPCK, PMSF, and antipain, prevent the loss of antigenicity, whereas others i.e., aprotonin, pepstatin, leupeptin, and TLCK do not. This suggests that the destruction of the Alz-50 epitope on A68 is due to ATP-activated proteolysis. Two monoclonal antibodies specific for A68, i.e., 42 and 30, protect the antigen from the proteolysis induced by ATP. Since a variety of proteins are degraded by an ATP\ubiquitin dependent pathway, an involvement of ubiquitin in the ATP-effect on A68 was examined. Exogenous ubiquitin partially protects A68 from degradation, whereas, polyclonal antibodies to ubiquitin prevent the loss of Alz-50 reactivity. In addition, hemin a specific inhibitor of ATP-activated proteolysis of ubiquitin-protein conjugates, abolishes the ATP-induced proteolysis of A68. Taken together, these findings indicate that the Alz-50 epitope is destroyed by a ubiquitin-mediated, ATP-dependent proteolytic system. This mechanism may be relevant to the physiological action of A68 in Alzheimer's disease or, may simply represent an attempt to abort an aberrant protein.

414.5

THE ISOLATION OF A RELATIVELY SPECIFIC PAIRED HELICAL FILAMENT ANTIBODY. S.G.Greenberg* and J.Schein (SPON: J.Pearson) Albert Einstein Coll.of Med.,Bronx, NY 10461

We have isolated a monoclonal antibody (PHF-1) from mice injected with denatured preparations of relatively nonaggregated populations of paired helical filaments (PHF). PHF-1 reacted with neurofibrillary tangles (NFT) and with neuritic processes of AD cases. Double labeling of AD sections revealed that the staining of NFT and neuronal processes was more extensive with PHF-1, than Alz 50. However, unlike Alz 50, PHF-1 did not label neonatal normal tissue. Immunoelectron microscopy demonstrated that PHF-1 reacted with PHF in PHF-enriched preparations. Like other PHF antibodies, PHF-1 recognized the 57-68 kd PHF proteins, their degradation products and unresolved higher MW material present in PHF-enriched preparations. While it is difficult to detect PHF proteins in homogenates with PHF antibodies such as Alz 50, PHF-1 appeared to react with PHF proteins in both homogenates and cytoskeletal enriched preparations from AD cases. Weak or no reactivity was observed with normal cytoskeletal proteins, including purified human tau. Thus, PHF-1 appears to recognize a unique epitope present on PHF. While the PHF-1 epitope may result from either a unique sequence or a posttranslational modification of a normal cytoskeletal epitope, the relative sensitivity and specificity of PHF-1 indicates that this antibody will be useful for the specific detection of relatively low levels of PHF and their component proteins during different pathological conditions. This work was supported by NIH grant #AG06803.

414.6

UNMASKING CRYPTIC EPITOPES OF PAIRED HELICAL FILAMENT IMMUNOREACTIVITY IN ALZHEIMER BRAINS USING FORMIC ACID. D.R. Sparkman, R.M. Ameen*, S.J. Hill*, C.L. White, III*. Neuropathology Laboratory, Department of Pathology, University of Texas Southwestern Medical Center, Dallas, Texas 75235-9072.

Formic acid (FA) pretreatment of tissue sections has been used to enhance immunostaining of beta amyloid (bA) deposits in Alzheimer disease (AD) brains. A monospecific antiserum (A128) to highly purified AD paired helical filaments (PHF) which does not immunostain the bA deposits in AD brain tissue sections was used on FA pretreated sections to assess whether contaminating antibodies to bA were present. Paraffin sections of AD brain rich in neuritic plaques (NP) were incubated overnight with anti-PHF antiserum A128 at serial two-fold dilutions ranging from 1:50 to 1:1600, or anti-bA antiserum A14-88 (1:25 through 1:800). Reactions were developed using the avidin-biotin immunoperoxidase technique with diaminobenzidine as the chromogen. Half of the sections were pretreated overnight with 99% FA prior to immunostaining. FA pretreatment enhanced the ability of NP to immunostain at higher dilutions with both anti-PHF and anti-bA antisera. Of particular note was the enhancement of PHF staining of NP around blood vessels. Preabsorption of the anti-PHF antiserum with purified amyloid from AD brain did not neutralize staining of these NP, whereas preabsorption of the anti-bA antiserum did. We conclude that 1) enhanced immunoreactivity of NP with anti-PHF antiserum A128 following FA pretreatment is not due to contaminating anti-bA antibodies, and 2) many perivascular NP previously interpreted as bA NP are, in fact, composed of PHF-rich neurites.

414.7

DECREASE IN NEURONAL MRNA IN ALZHEIMER DISEASE CORTEX CORRELATES WITH THE NUMBER OF NEUROFIBRILLARY TANGLES. I.M. Parhad, A.W. Clark, K.L. Goodison*, C.A. Robinson*. Pathology Dept., U. of Calgary, Alta, T2N-1N4, Canada.

Neuronal mRNA levels are decreased in the neocortex of Alzheimer disease (AD) brains. Case to case variation in AD neuropathology may reflect stages of AD degeneration. In this study we asked at which stage the mRNA decrement first appears. Seventeen AD cases (60-91 yrs) and 13 controls (55-82 yrs) were selected. The AD cases had varying severity of neuropathological changes: neurofibrillary tangle (NfT) counts in the frontal cortex ranged from 0-52/mm^2, and neuritic plaques (NP) ranged from 5-84/mm^2. The frontal or parietal cortex was processed for quantitative Northern analysis with cDNAs for neurofilament light subunit (Nf-L) and amyloid precursor protein (APP). Our results show an overall decrease of 40% for the Nf-L and APP mRNAs in the AD cases. The Nf-L mRNA inversely correlated with the number of NfT (r: -0.70, $p<0.01$), especially in the older cases (>70 yrs). The APP mRNA also had an inverse correlation with the number of NfT (-0.49) but this did not reach statistical significance. No correlation was seen between the numbers of NPs and Nf-L or APP. We interpret these results to mean that in senile dementia of Alzheimer type, the decrease in neuronal mRNA in the cortex occurs with the appearance of NfTs.

414.8

INTIAL STAGES OF TANGLE FORMATION IN DEGENERATING NEURONS OF AGED AND ALZHEIMER PATIENTS. M.A. Morán & P. Gómez-Ramos Dept. Morfología.Fac Medicina Univ. Autónoma Madrid.España.

In the brain of aged controls we studied the morphology of tangles in several cortical regions where these lesions appear first. We consistently found delicate Thioflavine-S positive threads, which were arranged as proximal dendrites surrounding a Thioflavine negative perikaryon full of lypofucsin. We also observed slightly thicker threads in the neuropil, somo of them in continuity with a neuronal perikaryon that had the appearance of early tangles. Next to mature tangles there were thick strongly fluorescent neurites, some of them in direct contact with a tangle. Adjacent sections stained for cholinesterases (AchE and BchE) showed positive reaction in early and mature tangles and only in the thickest neurites. Similar images were observed in selected isocortical areas of Alzheimer' brains.

We suggest that the sequence in the evolution of tangle formation may be as follows: 1) Amyloid starts to accumulate as delicate threads within proximal dendrites that are negative for cholinesterases. 2) Next, the perikaryon gets involved too. At this stage, staining for AchE and BchE parallels Thioflavine fluorescence. 3) Finally, mature old tangles appear to broke into thick strongly fluorescent neurites, some of which are positive for cholinesterases. The begining of AchE and BchE accumulation may represent the threshold in the degeneration process when transport is interrupted, and these enzymes are trapped among the altered cytoskeleton. Supported by FISSS 88/922 and 89/635.

414.9

ALZHEIMER SENILE PLAQUES, DYSTROPHIC NEURITES AND NEUROFIBRILLARY TANGLES STAIN POSITIVELY WITH ANTIBODIES TO THE CLASSICAL COMPLEMENT PATHWAY. P.L. McGeer, H. Akiyama* S. Itagaki* and E.G. McGeer. Kinsmen Lab. of Neurol. Res., Univ. British Columbia,2255 Wesbrook Mall, Vancouver, B.C., V6T 1W5, Canada.

We and others (1) have previously reported evidence of chronic inflammation in Alzheimer brain tissue. This includes the presence of HLA-DR positive reactive microglia, and T4 and T8 lymphocytes. We now report positive staining of senile plaques, dystrophic neurites and some neurofibrillary tangles with antibodies to several components of the classical, but not the alternative, complement pathway. Positive staining of these elements of Alzheimer brain tissue was obtained with polyclonal antisera directed against C1q, C3d and C9. Positive staining was also obtained with mouse monoclonal antibodies directed against C4d, C7 and a neoantigenic site on C5b-9. Monoclonal antibodies directed against the specific, alternative complement pathway factors, fraction Bb of factor B and factor P, failed to stain Alzheimer tissue. These data, indicating the presence of the full spectrum of complement components of the classical pathway, including the membrane attack complex, in affected elements of Alzheimer brain tissue, are further evidence of chronic inflammation in this disease.

1) J. Rogers et al, Neurobiol Aging 9:339-49, 1988.

414.10

ALTERATIONS IN LOCALIZATION OF Ca^{+2}-ATPase IN ALZHEIMER DISEASE BRAINS. B. Sisken, W. Markesbery* and T. Vanaman*. Center of Biomedical Engineering, Departments of Anatomy and Neurobiology; Pathology and Sanders Brown Center on Aging; Department of Biochemistry, University of Kentucky, Lexington, KY 40506.

Calcium metabolism alterations are of major interests in Alzheimer disease (AD) since abnormally high levels of internal free calcium are associated with neuronal degeneration. Two classes of Ca^{+2}-pumping ATPases which maintain low intercellular levels of calcium exist; at the cell surface and in the internal membranes of the cell. We have localized in normal and AD hippocami one distinct isoform of plasma membrane Ca^{2}-ATPase which we have previously identified by molecular cloning. In normal brain, astrocytes, oligodendoglia and microglia are non-reactive while ependyma, choroid plexus, arachnoid, pia and endothelial cells are reactive. Hippocampal dentate granule neurons, CA-4 neurons and some synaptic clusters are positive; CA-3 neurons are highly reactive while CA-2 CA-1 and subiculum neurons are progressively less immunoreactive; the molecular layer of the dentate gyrus is nonreactive. By contrast in AD the proximal, middle and distal molecular layer of the dentate gyrus are positive. CA-4 neurons, dendrites and large granular clusters of synapses show pronounced immunoreactivity while CA-3 through CA-1 neurons are similar to that in normals. Reactive astrocytes show marked immunoreactivity; neurofibrillary tangle-bearing neurons and senile plaques are nonreactive. These findings suggest altered calcium metabolism in reactive astrocytes and commissural, associational and entorhinal fiber pathways in the AD hippocampus. NS21868, 5PO1-AG05119, 5P50-AG05144.

414.11

EXAMINATION OF THE 5'END OF BRAIN MESSENGER RNA IN ALZHEIMER'S DISEASE. E.M. Sajdel-Sulkowska and E.S. Burnside.* Harvard. Med. Sch., Ma. Gen. Hosp., Boston, Ma. 02115 and McLean Hosp., Belmont, Ma. 02138.

We examined possible translational differences between Alzheimer and control mRNA from postmortem brains. The focus was on the 5'cap structure of the mRNA since it is involved in the initiation step of protein synthesis. The translational activity of mRNA isolated from the control (n=7) and AD (n=7) cases was measured in the rabbit reticulocyte system in the presence and absence of the cap analogue 7methylguanosine 5'triphosphate (pppm^{7}G). In the absence of pppm^{7}G control mRNA showed a 45% increase in the incorporation of ^{35}S methionine ($1.6x10^6$ cpm/ug mRNA) as compared to AD mRNA ($1.1x10^6$ cpm/ug mRNA). The cap analogue inhibited translation of mRNA in controls by 26% and in AD cases by 13.6% Mouse mRNA translated under identical conditions ($2.8x10^6$ cpm/ug mRNA) showed a 38.4% reduction in the presence of pppm^{7}G. These results indicate that the AD mRNA may be relatively undercapped as compared to control mRNA and are consistent with the reduction in the protein synthetic activity of AD mRNA in vitro and in living patients afflicted with AD. Supported by AHAF.

414.12

RELATIONSHIP OF NEURODEGENERATION TO THE EXPRESSION OF ALZHEIMER-ASSOCIATED ANTIGENS IN CULTURED HIPPOCAMPAL NEURONS. J. Mattson* and M. P. Mattson (SPON: M. B. Nikitovitch-Winer). Sanders-Brown Center on Aging and Dept. of Anatomy & Neurobiology, Univ. of Kentucky Med. Center, Lexington, KY.

Alzheimer's disease is characterized by the abnormal accumulation of several proteins in selectively vulnerable brain regions, including the hippocampus. Antibodies that recognize neurofibrillary tangle (NFT)-associated antigens (5E2 and Alz-50) or ubiquitin were used to examine the relationship between the presence of Alzheimer-associated antigens (AAAs) and neurodegeneration in cultured rat hippocampal neurons. In untreated cultures 5E2 antigen was expressed in most neurons with considerable variability in staining between neurons. Low to moderate levels of Alz-50 immunoreactivity were present in about 50% of the neurons. All neurons expressed low levels of ubiquitin. Subtoxic levels of glutamate or K^+ increased the expression of 5E2 and Alz-50 antigens but did not alter ubiquitin expression. Glutamate and K^+ at levels that caused a progressive degeneration of neurons over a 24 hr period induced increases in the expression all three AAAs, particularly in degenerating neurons. There was a striking increase in ubiquitin levels only in degenerating neurons. Higher levels of glutamate which caused rapid cell death (2-4 hrs) also increased AAA expression. Cycloheximide did not prevent induced increases in AAA levels suggesting that the increased levels of AAAs might reflect the modification of existing proteins. Finally, a subpopulation of neurons that was resistant to glutamate neurotoxicity did not express detectable levels of Alz-50 suggesting that Alz-50 antigen is a marker for neurons vulnerable to glutamate neurotoxicity. Collectively, these results suggest that the increased presence of at least some AAAs can result from excitatory overactivity, and are consistent with a model in which normal proteins are modified by the activation of neuronal signal transduction systems. The data are also consistent with the involvement of glutamate in Alzheimer's neuropathology. (Supported by the French Foundation for Alzheimer's Disease).

414.13

GANGLIOSIDES PREVENT RECOGNITION OF BRAIN ANTIGENS BY SERUM ANTIBODIES FROM AGED MICE.

H. Lal, E.N. Ahanotu*, V. Apte* and M.J. Forster. Department of Pharmacology, Texas College of Osteopathic Medicine, Fort Worth, TX 76107-2690.

Gangliosides have been shown to protect against several types of central nervous system (CNS) tissue injury (Mahadik & Karpiak, *Drug Dev. Res.*, 15:337, 1988), suggesting that they could be useful in treatment of degenerative CNS disorders. Because immunologic injury could contribute to age-related CNS disorders (Lal & Forster, *Neurobiol. Aging*, 9:733, 1988), we investigated the potential for gangliosides to prevent recognition of brain antigens by autoantibodies. Sera from a subset of aged (26-30 months) C57BL/6NNia mice contained IgG3 recognizing 64kD and 45kD proteins in brain cell membrane extracts. To determine the effect of gangliosides upon IgG binding, brain cells were pre-incubated with monosialoganglioside (GM1) or mixed gangliosides (0, 1, 5, or 10 $\mu g/10^4$cells) for 60 min, washed, and incubated with sera from old mice which had previously exhibited "brain-reactive" IgG3. When probed with peroxidase-conjugated, goat antimouse IgG, only the cells incubated with saline or 1 µg gangliosides showed immunoreactivity. These findings suggest that ganglioside treatments might protect CNS neurons from immunologic injury. [Supported by NIH grants AG06182 (MJF), AG07695 (HL), and NIH-BRSG grant RR05879].

414.14

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

We have demonstrated presence and characteristics of numerous immune system markers in human brain, several of which are differentially expressed in Alzheimer's disease (AD). The more difficult question remains, however, whether the appearance of these markers is merely an epiphenomenon of the disease process or has functional pathogenerative significance. We present several lines of evidence that the latter may be the case. Studies using antibodies directed against the Fc portion of human immunoglobulins reveal labelled neurons in all AD brains tested, but only a few normal elderly brains. In AD, many of the labelled neurons appear dystrophic, with fine Thioflavin S positive filaments coursing in the perikaryon. Since it seems unlikely that neurons produce antibodies, we suggest that the antibodies have bound to neurons, with the expected pathogenic consequences of antibody binding to a targeted antigen (e.g., complement proteins have been demonstrated in AD brain, especially in the context of neuritic plaques). The expression of cytokines is also a functional measure of immune activity as opposed to mere immune presence. Our studies show that several cytokines, particularly IL-1 and TNF, are differentially expressed in AD brain. IL-1 has recently been suggested to stimulate expression of B-amyloid (D. Goldgaber, personal communication). Lastly, our quantitative studies of immune system markers such as HLA-DR reveal a universal co-localization with neuritic plaques, within which levels of immunoreactivity are elevated as much as 50-fold compared to normal neuropil. Taken together, these and other data to be presented strongly suggest that whatever the etiology of AD, neuroimmune mechanisms contribute importantly to its pathogenesis. Supported by NIA AGO 7367-01A1 (JR).

414.15

CELLULAR AND MOLECULAR CHARACTERIZATION OF THE HUMAN MAJOR HISTOCOMPATIBILITY COMPLEX ANTIGEN HLA-DR IN ALZHEIMER'S DISEASE. W.H. Civin, S.D. Styren, S.A. Allen, and J. Rogers. Institute for Biogerontology Research, Sun City, AZ 85351.

Despite reports of an absence or paucity of CNS immunoreactivity for major histocompatibility complex (MHC) antigens in normal humans and laboratory animals, we suggested several years ago that MHC type II antigens such as HLA-DR, -DP, and -DQ are abundantly expressed in white matter of normal elderly patients and white and gray matter of Alzheimer's disease (AD) patients (Rogers et al., Neurosci. Abs., 12:944, 1986). Our data have since been replicated and extended by McGeer and associates (McGeer et al., Neurosci. Lett., 79:195, 1987). Crucial questions remain, however, as to the exact molecular identity of the brain protein labelled by HLA-DR immunohistochemistry, and the cell types which express that protein in brain. We have performed Western blot analysis, and light and electron microscopic immunohistochemistry to resolve these issues. Our HLA-DR antibody detects a protein in AD and normal elderly brain with the expected molecular weight for HLA-DR (alpha chain), and which runs parallel to the HLA-DR immunoreactive band from lymph nodes on Western blots. At the light and electron microscopic level, HLA-DR immunoreactivity is detected on cells with the morphologic and ultrastructural characteristics of astrocytes, microglia, and perivascular cells. Neurons are never stained. Double-labelling studies confirm these observations. The majority of microglia in AD brain are HLA-DR positive, whereas HLA-DR immunoreactive astrocytes are relatively rare. HLA-DR immunoreactivity co-localizes universally with the neuritic plaques of AD, but not necessarily with neurofibrillary tangles. Supported by the Arizona Disease Control Research Commission (JR).

414.16

TRANSFERRIN AND FERRITIN IN THE HUMAN BRAIN IN NORMAL AGED AND ALZHEIMER'S DISEASE. James R. Connor[1], Sharon L. Menzies[1]*, Richard E. Fine[2], and Elliott J. Mufson[3]. [1]Dept. of Anatomy, M.S. Hershey Medical Center, Hershey PA, 17033, [2]GRECC Unit, ENR Veterans Hospital, Bedford MA and [3]Institute for Biogerontology Research, Sun City AZ.

Our approach to studying CNS iron regulation has been to focus on transferrin (Tf) the major iron mobilization protein and ferritin, the major iron storage protein. In the present study, 2 cerebral cortical areas were examined immunohistochemically in tissue obtained at autopsy from 8 normal adult human brains (non-neurologically related cause of death) and 5 patients with Alzheimer's disease (AD). Tf immunoreactivity (Boehringer-Mannheim; 1:250) in both the normal and AD tissue was predominantly confined to oligodendrocytes. Some cells in the white matter which appeared to be astrocytes were immunoreactive in the AD tissue. Ferritin (ICN immunobiologicals, 1:500) immunoreactivity is specific to oligodendrocytes in normal brain tissue. Some ferritin immunoreactive cells are found in association with senile plaques and these cells may be macrophages or microglia. Quantitative analyses of Tf and ferritin are in progress. The results of this study suggest that oligodendrocytes may play a role in iron regulation in the brain.
Supported by Alzheimer's Disease Research, a program of American Health Assistance Foundation.

414.17

LACK OF ALUMINUM IN ALZHEIMER'S DISEASE (AD) BY "SMALL SAMPLE" FLAMELESS ATOMIC ABSORPTION ANALYSIS. R.W. Jacobs, G.A. Trapp, T. Duong and A.B. Scheibel, Depts. of Anatomy, Psychiatry, and the Brain Research Inst., Univ. of California, Los Angeles, CA 90024; and VA Medical Center and Dept. of Psychiatry, Univ. of Texas Southwestern Med. School, Dallas, TX, 75216.

We previously reported no detectable aluminum (Al) levels in seven AD and four control cases by X-ray microprobe analysis (Jacobs et al., Soc. Neurosci. Abstr., Vol. 14, Part 2, p.1083, 1988). We have attempted to verify these negative findings by the use of atomic absorption spectrophotometry (AAS). Fresh frozen, coronal sections from six of the seven AD (mean 80 yrs; range 68-102) and four control brains (mean 66 yrs; range 58-78) used in the previous study were obtained from the National Neurological Research Bank, Wadsworth VA Medical Center. Samples (ranging from 48-144 mg wet wt.) from Brodmann areas 9, 11, 28, 46, 47, and hippocampus were wet-ashed for atomic absorption analysis and an NH_3NO_4 matrix modifier to eliminate chloride interference on aluminum (Trapp, G.A., Anal. Biochem., in press). The remaining tissue surrounding each sample site was histologically prepared for thioflavin-S, and characterized for intensity of plaques and tangles. Our results show a range from undetectable to 1.80 ng/mg wet wt. [mean, 0.54 ± 0.58 (SD)] of Al in control tissue. AD samples revealed similar Al levels ranging from undetectable to 1.76 ng/mg wet wt. [mean, 0.28 ± 0.39 (SD)] independent of histopathology. There was also no correlation between age and Al levels suggesting that Al may not accumulate over time in the CNS. The confirmation of no abnormal amounts of Al by AAS in our AD specimens suggests that this metal may be unrelated to the pathogenesis of plaques and tangles in AD.

414.18

ALUMINUM ACCESS TO THE BRAIN: A POSSIBLE ROLE FOR TRANSFERRIN. A. Jane Roskams and James R. Connor. Center for Neuroscience and Department of Anatomy, M.S. Hershey Medical Center, Hershey PA 17033.

Transferrin (Tf), the iron mobilization protein from plasma utilizes a receptor on brain endothelial cells to transport iron across the blood-brain-barrier. In addition to iron, Tf binds aluminum. Indeed, as much as 30% of the Tf in plasma is bound to aluminum. Two studies are described here: 1) Using 125I-Tf on brain membrane preparations we found that the brain Tf receptor has a Kd of approximately 0.5nM; 2) We established that the Tf-aluminum complex behaves similar to Tf-iron in its interactions with the Tf receptor. Tf-aluminum ($Tf\text{-}Al^{+3}$) is prepared by incubating iron-free Tf (Sigma) with $AlKSO_4$ in the presence of bicarbonate (pH 7.4) and the $Tf\text{-}Al^{+3}$ complex is demonstrated spectrophotometrically. Direct competition assays were performed with 125I-Tf (0.25, 0.5 and 1.0nM) in the presence of an excess (5.0um) of either Tf-iron, $Tf\text{-}Al^{+3}$ or iron-free (Apo-Tf)Tf. Tf-iron and $Tf\text{-}Al^{+3}$ competed equally for the Tf receptor; whereas Apo-Tf was only 33-50% as effective. Following incubation with saturating concentrations of 125I-Tf, both $Tf\text{-}Fe^{+3}$ and $Tf\text{-}Al^{+3}$, but not Apo-Tf (5.0um) were able to displace 100% of bound 125I-Tf. These results suggest that aluminum could use the Tf-Tf receptor system to gain access to the CNS. This work is supported by the American Federation for Aging Research.

414.19

VISUALIZATION OF NON-MICROTUBULE TAU BINDING PROTEINS BY LIGAND BLOTTING. D. M. Wilson* and L. I. Binder* (SPON: L. E. Mays). Dept. of Cell Biology and Anatomy, Univ. of Alabama at Birmingham, 35205.

Understanding the normal in vivo function(s) of tau and those properties of tau which may lead to abnormal aggregation states, would be enhanced by a knowledge of the full repertoire of proteins capable of interacting with this microtubule associated protein (MAP). Towards this end we have developed a ligand blotting assay to identify possible tau binding proteins. The procedure involves incubating nitrocellulose bound proteins with purified bovine tau, followed by detection of bound tau with anti-tau mAb's. Incubation of rat cortex homogenates with 0.7 uM tau reveals high affinity binding to a triplet of proteins in the 100-130 kD range. These proteins are distinct from tau or tubulin dimers also seen in this range. Two of these proteins are enriched in preparations of taxol stabilized microtubules. Several protein bands are bound with lower apparent affinity; among these are tubulin, MAP1b, and MAP2. Work is underway to determine the prevalence of the high affinity tau binding proteins in human brain tissue of normal and Alzheimer's disease origin.

414.20

IN VITRO STUDIES OF THE INTERACTION BETWEEN THE ALZHEIMER COMPONENTS α_1-ANTICHYMOTRYPSIN AND β-PROTEIN. D. Dressler*, C.R. Abraham, P. Amsterdam* and H. Potter. Dept. Neurobiology, Harvard Med. Sch., Boston, MA 02115.

The protease inhibitor α_1-antichymotrypsin (ACT) has been shown to be an integral component of the amyloid deposits of Alzheimer's disease and the similar deposits arising in Down's syndrome and in normal aging in humans and monkeys. ACT was also found associated, together with the β-protein, in one non-Alzheimer amyloid—a variant of hereditary cerebral hemorrhage with amyloidosis found in Holland (Abraham et al., submitted). Together these results indicate that there is a special association of ACT and the β-protein, perhaps essential to the formation of amyloid. We are therefore examining the interaction between ACT and the β-protein *in vitro*. A radioiodine-labeled peptide corresponding to amino acids 1-28 of the β-peptide becomes bound to ACT in an SDS-resistant complex. The use of cross-linking agents stabilizes even a greater amount of the peptide onto ACT. Other experiments suggest that the 1-28 peptide may bind to the ACT protein at its active protease-inhibitory site: (1) the addition of chymotrypsin to ACT prior to the addition of the peptide prevents the association and (2) the peptide prevents ACT from being able to subsequently inhibit chymotrypsin. An examination of the amino acid sequence of the β-peptide reveals a region near the N-terminus, which shows striking homology to the active site of serine proteases. Studies with peptides having specific amino acids changed in this region are underway to test whether this protease-like region is the basis for the specific association of ACT and the β-protein.. A model for the Alzheimer-like amyloid filaments based on this interaction will be presented.

414.21

Polypeptide fragments derived from insoluble components purified from Alzheimer, Pick's and Parkinson's diseased cortex. G.D. Vogelsang[1], G.E. Dean[2]*, and F.P. Zemlan[3]. Depts. of [1]Physiology, [2]Molecular Genetics and [3]Psychiatry, Univ. of Cincinnati College of Medicine, Cincinnati, OH 45267.

Paired helical filaments (PHF) were electrophoretically purified from high density neurofibrillary tangle (NFT)-bearing Alzheimer (AD) parietal cortex. Straight filaments were isolated from identically prepared Pick's and Parkinson's brain tissue lacking NFT. Subsequent electrophoresis solubilized these relatively insoluble structures. Silver-stained protein profiles of these solubilized structures appeared identical on sodium docecyl-sulfate polyacrylamide gels. The predominant polypeptide fragments were limited to M_r ~55-66 kilodaltons. Identically derived material from normal brain tissue lacking NFT's had a similar protein profile, but only amorphous aggregates were observed in the material solubilized. PHF and straight filaments were equally insoluble, but straight filaments had a smaller diameter (≤ 10nm) and lacked the helical nature of PHF. Only the AD derived material had demonstrated PHF-specific immunoreactivity as judged by Western blot analysis. Actin immunoreactivity was indicated at the M_r ~64-66 kilodalton range. The other brain types also displayed a reduced amount of this unusual actin-immunoreactivity/ug protein at the exact molecular weight range. It appears that the proteins associated with PHF structure may be common to all brain types and the inherent insoluble components that can be derived from each, regardless of their ultra-structure. The feature which causes AD-specific immunoreactivity may be attributed to a conjugation or modification of these common proteins.

414.22

SUBCELLULAR THREE-DIMENSIONAL ANALYSES OF HUMAN CORTICAL PYRAMIDAL NEURONS WITH ALZHEIMER PATHOLOGY BY HIGH VOLTAGE ELECTRON MICROSCOPY AND COMPUTER-AIDED RECONSTRUCTIONS FROM SERIAL SECTIONS. S. J. Young, D. Hessler*, T.J. Deerinck, and M.H. Ellisman (SPON: J.M.LeBeau). Lab. for Neurocytology, U.C.S.D., LaJolla, Ca. 92093.

We have been studying alterations in the form, content and spatial distribution of subcellular elements of cortical pyramidal neurons obtained from human biopsy specimens exhibiting the pathology typical of Alzheimer's disease. We have compared neurons differing in pathology, as indexed by the amount of paired helical filament present. Differences between neurons were examined using (1) computer-assisted three-dimensional reconstruction of electron micrographs from serial sections, and (2) stereo-pair views of thick sectioned tissue obtained with the high voltage electron microscope. Series of 50-100 .25μm serial sections were obtained through selected neurons. The serial reconstruction was derived from digitized outlines of membrane bounded structures including the plasma membrane, the nucleus and the Golgi apparatus and line segments representing paired helical filaments (PHF). Digitization and generation of preliminary views, in the form of simple hidden line reconstructions, was performed on a personal computer. The digitized data was then ported to an Ardent Titan graphics mini-supercomputer to apply programs providing rapid computation and display from different orientations, with cut-away views of interior structure, employing sophisticated reconstruction and rendering techniques. These reconstructions, in conjunction with stereo-views from high voltage thick sectioned images, have revealed possible adaptive changes in the cytoplasmic organization and possible mechanisms underlying neuronal dysfunction in Alzheimer's disease. In contrast to neurons without PHF, pathological neurons display associations of the PHF with the membranes of the nuclear envelope and displacements of the Golgi apparatus. Supported by ADRDA IIRG88-106, PHS RR04050/RR00592.

414.23

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

Some theories explain the pathogenesis of Alzheimer's disease (AD) as the result of a decrease in the production of growth factors (GF's). Other studies, however, suggest a sprouting reaction in AD probably mediated through GF's. To get further insight into the involvement of GF's, we performed immunohistochemical studies of proteins that provided an index of GF stimulation, (eg: fos, PKC). We found, by comparing five AD and five control cases, a 40% decrease in the number of immunoreacting neurons in the AD nucleus basalis with anti-fos and anti-PKC α, βII and γ. In contrast, the neocortex and hippocampus of AD cases presented an increase in the number of immunostained neurons with anti-fos as well as immunolabeled neurites in the plaques with anti-PKC α, and βI. These data suggest a possible decrease of GF's stimulation in subcortical areas, probably related to the hippocampal deafferantation with an increase in the GF stimulation in the hippocampus and neocortex possibly in relation to compensatory sprouting reaction.

414.24

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

Epidermal growth factor receptor (EGFR) is a 170 KD integral membrane protein. It contains a tyrosine kinase moiety, and is activated by the binding of epidermal growth factor, transforming growth factor and vaccinia virus growth factor. EGFR binding results in increased mitotic activity and upregulation of cytoplasmic signalling pathways within the target cell. We have utilized light and EM immunohistochemistry as well as Western blot analysis to examine EGFR expression in the brains of nondemented and clinically demented patients (Alzheimer's disease, Parkinsonian dementia, multi-infarct dementia). Immunohistochemical analysis of EGFR distribution within these groups demonstrates a correlation between presence of dementia and the expression of EGFR. Ten patients with a variety of neurological dementia were positive for EGFR expression throughout all regions of brain examined. Eight patients without dementia did not exhibit any EGFR immunoreactivity. One patient without clinical diagnosis of dementia but with extreme Alzheimer's pathology reacted positively for EGFR, as did a nondemented patent with advanced malignancy of the spinal cord. In affected patients analysis of immunoreactivity at the ultrastructural level confirms the presence of EGFR on the lumenal surface of the endothelial cells lining the blood vessels. In addition to the vascular staining, preliminary analysis also shows EGFR immunoreactivity within the cytoplasm of glial cells. Based on these observations we conclude that EGFR expression 1) is associated with the presence of clinical dementia, 2) is present throughout the brain once induced, 3) is predominantly localized to the blood vascular endothelial cells of affected patients, and 4) may be inducible by factors common to physiological states which induce dementia. Supported by NIA AGO 7367-01A1 (JR).

414.25

Effects of Nerve and Epidermal Growth Factors on the Metabolism of the Alzheimer Amyloid Precursor in Cell Cultures. Lawrence M. Refolo*, Steven R.J. Salton, John P. Anderson*, Pankaj Mehta* and Nikolaos K. Robakis, Department of Psychiatry and Neurobiology, Mount Sinai School of Medicine, New York, NY 10029

Antisera against specific regions of the Alzheimer βAmyloid protein precursor (βAPP) were used to study the effects of nerve and epidermal growth factors on the expression and processing of this protein in PC12 cell cultures. Two major βAPP proteins containing the Kunitz-protease inhibitor (KPI) were detected in cell extracts of naive PC12 cells. One displayed an Mr of 100,000 and the other displayed an Mr of 145,000. Addition of nerve growth factor (NGF) to PC12 cell cultures resulted in a large decrease in the amount of the 100,000 kDa protein and in an increase in the amount of the 145,000 kDa protein. No detectable change was seen in the levels of βAPP mRNA. Medium from naive PC12 cell cultures contained minimal amounts of either the 145,000 or the 100,000 kDa βAPP. Treatment of these PC12 cultures by NGF resulted in the release to the medium of high levels of a 125 KDa βAPP form which derives from the KPI containing βAPP and lacks the carboxyl-terminal part of the precursor. Lectin chromatography revealed that the secreted βAPP consists of at least two different glycosylated forms. The cellular form of βAPP contains both N-linked and O-linked sugars, while the forms released in response to NGF treatment contain only O-linked sugars. Epidermal growth factor (EGF) also induced the release of a βAPP protein in the culture medium, although this factor had no specific effect on either the levels or mobility of the cell-associated form of βAPP. The level of the cellular 145,000 KDa form of βAPP increases with cell density, suggesting that the expression of the KPI-containing βAPP may be modulated by intercellular contact.

414.26

THE ALZHEIMER'S RELATED HIPPOCAMPAL cDNA CLONE pADHC-9 ENCODES A 52 kDAL PROTEIN SIMILAR TO A RAT TESTICULAR SULFATED GLYCOPROTEIN. P.C. May, M.A. Lampert-Etchells*, C.P. Anderson*, and C.E. Finch. Andrus Gerontology Center, Dept. Biol. Sciences, USC, Los Angeles, CA 90089.

Clone pADHC-9 encodes a 2Kb RNA which is overexpressed in Alzheimer's disease hippocampus. pADHC-9 was isolated by differential screening of a mixed Alzheimer's/Control hippocampal cDNA library. Nucleotide sequencing of pADHC-9 identified an open reading frame of 449 amino acid residues coding for a putative 52 kDal protein. To confirm this reading frame, ^{7me}GpppG-capped RNA was transcribed in vitro and translated using a rabbit reticulocyte lysate system. A prominent 52 kDal protein was observed following translation indicating an authentic open reading frame of the appropriate size. The nucleotide and deduced amino acid sequence of this protein share 75-80% identity to rat sulfated glycoprotein-2, the main secretory product of Sertoli cells in the rat testes. A consensus signal peptide, 6 presumptive ASN-linked glycosylation sites, 10 CYS residues and a proteolytic cleavage site are conserved between these two proteins. These data suggest that pADHC-9 may encode a highly processed glycoprotein related if not identical to a human analogue for rat sulfated glycoprotein-2; its function in brain is unknown. Supported by ADRC Grant # AG005142, the John D. and Catherine T. MacArthur Foundation Research On Successfull Aging(CEF) and ADRDA Grant IIRG-88-069 (PCM).

414.27

DEVELOPMENT AND CHARACTERIZATION OF SIX NEW MONOCLONAL ANTIBODIES THAT BIND TO ALZHEIMER'S DISEASE ASSOCIATED PROTEINS (ADAP) H. A. Ghanbari*, S. Riesing*, B. E. Miller, I. Tribby* (SPONS. C. Harrington) Mental Illness and Neurological Diseases Diagnostics, Abbott Laboratories, Abbott Park, IL 60064

We have developed and produced 6 new monoclonal antibodies (MAb's) that selectively bind to a group of proteins (isoforms) which appear as 3 major bands (53, 61, and 67 kDaltons) on SDS-PAGE/Western Blots using ALZ50 monoclonal antibody. These proteins, refered to as ADAP, are present in Alzheimer Disease (AD) and some Down's Syndrome brain tissue and absent in normal and other neurological disease specimens. The new MAb's have been produced against a highly ADAP-enriched fraction prepared by multi-step differential centrifugation and detergent extraction of AD brain homogenates. These antibodies (all IgG's) share the antigen (ADAP) with ALZ50 but not necessarily the epitope. Two of the antibodies, ADD-11 and ADD-38 have much higher affinity for ADAP than ALZ50 but demonstrate the same pattern of cross-reactivity against normal brain components by both ELISA and Western Blot. The antibodies ADD-9 , ADD-14, ADD-29 and ADD-36 however demonstrate minimal cross-reactivity with normal tissue extracts, particularily in the tau region of the Western Blots. An Alzheimer's test is being developed using these new antibodies.

414.28

ALZHEIMER-LIKE PATHOLOGY INDUCED BY HUMAN SERUM IN CULTURED HIPPOCAMPAL NEURONS. G.J.Brewer, L.Domino*, and J.W. Ashford. Southern Illinois Univ., Springfield, IL 62794.

Toxic or immunologic factors in human serum could contribute to the pathogenesis of Alzheimer disease(AD). To test this theory, we grew embryonic rat hippocampal neurons in a serum-free culture medium that supports growth of neurons but not glia at low density (Brewer & Cotman, Brain Res., in press). To test for the induction of AD-like changes, cultures grown for 4 days were treated with 10% human serum. After 24 hr., neurons were fixed, one of three AD-specific stains were applied, and digitized fluorescence intensities were recorded. 1) Thioflavin S binds to the β-amyloid found in AD plaques. In culture, 10 of 12 sera tested produced significantly more fluorescence than untreated neurons. Fluorescence was particularly strong among clusters of cells. 2) Alz-50 obtained from P. Davies is a monoclonal antibody (mAb) generated by immunization with an AD brain homogenate. 14 of 14 sera produced greater immunofluorescence either in the soma or processes compared to untreated neurons in which only minimal fluorescence was found. 3) a mAb specific for the β-amyloid in AD plaques obtained from Kim & Wisniewski produced immunofluoresceence in neurons treated with sera as follows: one AD serum >> one normal serum > no serum. These results show that a serum factor can induce AD-like neuropathology, which in the AD brain may be aided by a compromised blood-brain barrier. Funded by IDPH and the Pearson Foundation.

414.29

SELECTIVE DISCONNECTION OF SPECIFIC VISUAL PATHWAYS IN CASES OF ALZHEIMER'S DISEASE COMBINED WITH BALINT'S SYNDROME. P.R. Hof, C. Bouras*, J. Constantinidis*, and J.H. Morrison, Research Institute of Scripps Clinic, La Jolla, CA, and Dept of Psychiatry, Univ. of Geneva, Switzerland.

A quantitative neuropathological analysis was performed in a subpopulation of Alzheimer's disease (AD) patients that presented a visual defect referred to as Balint's syndrome (ADB) early in the course of the disease. Balint's syndrome is a defect in visuospatial skills and the distribution of pathology in the ADB cases suggests that the connections subserving this component of the visual system are devastated, whereas they are usually spared in AD. In area 17, neurofibrillary tangle (NFT) density in the ADB cases as compared to the AD cases increases up to 63-fold. A similar increase of up to 16-fold in area 18 and 8.9-fold in area 19 was demonstrated. NFT counts were significantly lower (50%) in the prefrontal cortex of ADB as compared to the AD cases. No differences in NFT density were observed in visual association area 20 of the temporal lobe. Neuritic plaques (NP) were more numerous in the occipital cortex of the ADB cases, with increases up to 3.4-fold. Area 9 showed less NP in ADB than in AD cases. Area 7 in the parietal lobe was characterized by increased NP densities (up to 2.4-fold) and larger NP in the ADB cases. No differences in NP density were noted in area 20. Finally, Meynert cell counts in area 17 were significantly lower (41%) in ADB. These data suggest that in some cases of AD, specific neurological symptomatology may be caused by the selective loss of specific corticocortical systems, as reflected by a differential distribution of the neuropathological markers of the disease. Supported by grants from NIA (AG06647) and FNRS (83.495.0.87).

414.30

DENDRITIC EXTENT IN HUMAN BASAL FOREBRAIN IN NORMAL AGING AND ALZHEIMER'S DISEASE. S. D. Hanks* and D. G. Flood (SPON: E. M. Grollman). Depts. of Neurology and Neurobiology & Anatomy, Univ. of Rochester Sch. of Med. & Dent., Rochester, NY 14642.

Previous studies of dendritic extent in normal aging have led to the suggestion that, contrary to long-accepted dogma, the adult human neuron is capable of a remarkable degree of plasticity. Evidence has accumulated that alteration or loss of this latent plastic capacity may be a pathophysiologic feature of Alzheimer's disease (AD). In the present study, dendritic extent was measured in human basal forebrain in normal aging and AD.

From over 80 cases obtained at autopsy through the Rochester Alzheimer's Disease Project (RADP), 12 subjects were assigned to each of the following two groups (6 per group): 1) normal aged adults (mean 81.7 years, range 79-84 years), and 2) AD (mean 81.8 years, range 77-87 years). All cases in the latter group had a history of dementia which proved to be AD at autopsy. All cases in the normal aged group lacked a history of any neurological or psychiatric abnormality and showed no significant neuropathology at autopsy. Fifteen neurons from each of three regions of the basal forebrain (Ch4am, Ch4al and Ch2, as defined by Mesulam, M.-M. and Geula, C., *J. Comp. Neurol.*, 275:216-240, 1988) were randomly selected for analysis from coded 200 µm sections of Golgi-Cox stained tissue. Camera lucida tracings of these cells were prepared and subsequently digitized and measured by an Apple II Plus microcomputer with attached graphics tablet. Computer-assisted analysis currently underway will provide comparative measures of cell body size and multiple parameters of dendritic extent, including total length, total number, and average length, for dendritic segments ordered in both a centrifugal and centripetal direction. These data will be compared with previous reports of dendritic extent in human basal forebrain as well as a variety of other brain regions. Supported by NIH grant AG 03644.

414.31

CALCIFICATION OF THE GLOBUS PALLIDUS FOLLOWING EXCITOTOXIC DESTRUCTION OF THE NUCLEUS BASALIS

G.R. Stewart, D.F. Wozniak, R.E. Schmidt, and J.W. Olney. Depts of Psychiatry and Pathology, Washington University School of Medicine, St. Louis, MO.

In studies aimed at clarifying the role of cholinergic degeneration in Alzheimer's disease, we have injected the excitotoxin, N-methylaspartate (NMA), into the rat nucleus basalis to destroy basal forebrain cholinergic (BFC) neurons. In long term survival experiments (up to 11 months post-lesion), we frequently found conspicuous mineralized deposits in the globus pallidus (GP). The deposits occurred in three forms: 1) large, translucent, spherical concretions; 2) smaller, more irregular-shaped deposits, sometimes with profiles resembling neurons; and 3) as a flocculant accumulation of material surrounding large blood vessels. Spherical concretions were the most common form encountered; these were quite variable with respect to the size and number of spherules and the extent of their areal involvement of GP. The longer the survival time, especially if it extended beyond 6 months, the more consistent and severe was the response. Applying mineral-specific stains, we found that the concretions contain calcium and iron.

Inducing GP deposits is not a property unique to NMA since other investigators, using a different excitotoxin (ibotenate) to produce BFC lesions, have noted similar GP deposits. It is puzzling that the location of these deposits and the placement of the BFC lesion do not precisely coincide. Typically, the deposits are absent from the center of the injection site where BFC cell loss is most severe, but are present ventrally and laterally in regions where there is substantial sparing of BFC neurons. Thus, it is unclear how these mineral deposits relate to the destruction of BFC neurons. However, their presence in relatively close proximity to a focus of excitotoxin-induced BFC neuronal degeneration is noteworthy, since basal ganglia calcification is occasionally found in human brain and reportedly occurs with increased frequency in Alzheimer's disease and Down's syndrome (Mann, 1988), both of which entail degeneration of BFC neurons. Supported by RSA MH 38894 (JWO), AG 05681 and ES 07066.

414.32

HYPOTHALAMIC-PITUITARY-ADRENAL FUNCTION IN ALZHEIMER PATIENTS AND AGED CONTROLS.

M.J. Meaney, M. Sharma, S. Sharma, M. Thakur, A. Issa, R. Quirion, R.M. Sapolsky, & N.P.V. Nair. Douglas Hospital Research Ctr., Dept. Psychiatry, McGill Univ., Montreal H4H 1R3, Canada and Dept. Biological Sciences, Stanford Univ., Stanford, CA 94305.

Basal hypothalamic-pituitary-adrenal (HPA) function appears unchanged in healthy, aged humans. However, HPA dysfunction is frequently associated with neuropathology in the aged, and the results of animals studies have indicated that increased HPA activity is selectively associated with hippocampal dysfunction and cognitive deficits among aged rats. Existing data suggest that increased basal HPA acitivity might accompany Alzheimer's disease (e.g. Greenwald, B. Am. J. Psychiatry, 1988).

We have examined basal cortisol levels in Alzheimer patients (n= 6) and same-aged, healthy controls (n = 12), implanted with indwelling catheters at 0700h. Samples were taken every hour for 25 h and cortisol levels were estimated using a radioimmunoassay (Krey et. al, Endocrinology, 1975). Plasma cortisol levels were significantly ($P<0.001$) elevated in Alzheimer patients during both the AM (9.6± 1.1 vs. 5.9±0.3 µg/dl/h) and PM (12.6± 0.7 vs. 8.7±0.4 µg/dl/h) phases of the cycle. The differences occurred during the mid to late AM and early PM time points. The two populations exhibited virtually identical curves for cortisol over the diurnal cycle, with peak cortisol levels achieved at about 0800-0900h. Preliminary data indicate a similar trend for ACTH.

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

414.33

THE EFFECT OF AGING ON NEURONAL LOSS IN THE ENTORHINAL CORTEX: A RELATIONSHIP TO ALZHEIMER'S DISEASE?

C.F. Lippa, J.E. Hamos and D.A. Drachman. Department of Neurology, University of Massachusetts Medical Center, Worcester, MA 01655.

To assess the effects of normal aging on the cells of origin of the hippocampal perforant path, large neurons comprising islands within layer II of the entorhinal cortex (ERC) were counted using a semi-automated image analysis system. Neuronal counts were obtained from different rostrocaudal levels of the ERC in 10 cognitively normal individuals of differing ages. To assess a possible relationship with Alzheimer's Disease (AD) we also evaluated 8 AD patients. Our data demonstrated that with aging, cognitively normal individuals lose neurons in the ERC and that this loss may become prominent in some aged individuals. As also reported by others, all AD patients we studied showed an extreme loss of ERC neurons. These results are compatible with the hypothesis that AD is an acceleration and/or exaggeration of a process that occurs in normal aging.

414.34

EXPRESSION OF HEAT SHOCK PROTEINS IN ALZHEIMER'S DISEASE.

J.E. Hamos, B. Oblas, D. Pulaski-Salo*, P.J. Apostolides* and D.A. Drachman. Dept. of Neurology, Univ. of Mass. Med. Ctr., Worcester, MA 01655.

Heat shock proteins (HSPs) are ubiquitous polypeptides expressed in response to a wide variety of cellular stressors. To investigate the changes of expression of these proteins during Alzheimer's Disease (AD), we examined medial temporal lobes of AD patients and age-matched controls.

Utilizing immunocytochemical techniques and monoclonal antibodies to two HSPs of M.W. 78Kd (D. Bole, Yale Univ.) and 72Kd (W. Welch, UCSF), we found that in control brains, HSP72 was present uniformly in neurons, glia, and neuropil. HSP78 antibodies stained neurons in the subiculum and CA1 intensely, and neurons and glia in the dentate gyrus, other hippocampal fields, and the entorhinal cortex moderately. Western blots confirmed the identification of these HSPs.

In AD brains, the pattern changed dramatically. HSP72 antibodies intensely stained neuritic plaques and neurofibrillary tangles. HSP78 was found only in cytologically normal neurons, and even increased in neurons in the entorhinal cortex and CA3. Double labeling with ALZ50 antibodies (P. Davies, Albert Einstein College of Med.), which stain plaques and tangles, showed that HSP78 did not colocalize with pathologically affected neurons.

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

414.35

MAGNETIC RESONANCE IMAGING OF THE HIPPOCAMPAL-ENTORHINAL CIRCUITRY IN ALZHEIMER'S DISEASE.

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The neuropathology of Alzheimer's Disease (AD) shows a consistent and severe loss of hippocampal (HPC) and entorhinal (EC) neurons. However, neural pathology is characterized at autopsy, making identification of neural degeneration during the disease difficult. MRI can provide a means to quantify anatomical changes in select brain regions, such as HPC and EC, during the course of AD and these changes can then be related to behavioral changes associated with the structural degeneration.

To date, 4 patients with mild to moderate AD and 4 age and sex matched normal volunteers have received MRI scans of the brains. Scanning was done on a GE Signa Unit, with 1.5 Tesla superconducting magnet and a head coil. Three series of scans were taken at each examination: *Spin Echo* -(1) Saggital T1 weighted: TR 600, TE 20, 256 x 256, 5mm slice thickness with 2.5mm gap, 1 excitation, 11 slices, field of view 22 cm. (2) Transverse, T2 weighted: TR 3700, TEs 100, 256 x 256, 5mm slice thickness with 2.5mm gap, 1 excitation, slices to cover the head, field of view 24 cm. *Inversion Recovery* - (3) Coronal: TR 2500, TI 900, TE 20, 256 x 256, 5mm slice thickness with 1 mm gap, t2 excitations, slices through temporal lobe, field of view 20 cm. The coronal IR images were analyzed to determine the volume within a 29 mm section of HPC and EC. Matched-pair t-tests indicated that the AD groups HPC and EC were significantly smaller than the sex and age matched control groups (respectively, $t = 15.07$, $p > 0.001$ and $t = 22.21$, $p > 0.001$).

Compared to their age matched controls, patients with AD showed a reduction of 51 % in HPC and 31 % in EC size. Hippocampal and entorhinal atrophy was highly correlated with the mini mental status exam, olfactory match-to-sample and smell identification tests and had a low correlation with a visual match-to-sample test. Thus, MRI appears to be a sensitive technique to quantify neural degeneration and functional correlates during the course of AD.

Supported by AGO 7918-01

414.36

SELECTIVE LOSS OF CENTRAL PATHWAYS FOLLOWING VIRAL INOCULATION IN THE RAT OLFACTORY SYSTEM.

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

In anaesthetized rats, 200 nl of 10^7 PFU/ml HSV1 (F strain) was injected into the olfactory bulb. Following survival of 3-5 days, rats were sacrificed by perfusion and frozen sections of the brain were processed by immunocytochemistry to visualize neurotransmitter or virus location. Following these short survival periods, virus-infected cells could still express neurotransmitter in cholinergic neurons of the diagonal band, serotonergic neurons in the raphe nucleus, and noradrenergic neurons in the locus coeruleus. With survival periods of 17 days to 3 months following viral inoculation, serotonergic and noradrenergic innervation of the olfactory bulb and some cortical regions was dramatically reduced. Cholinergic input to the olfactory bulb (determined by AChE histochemistry) was slightly reduced. These results compare well to the deficits observed in AD although potentially important differences were apparent. The present results may also provide insights into the etiology of AD. Supported by the American Health Assistance Foundation, NIH NS 23348 and NIAID AI-23482.

414.37

OCCURRENCE OF DIFFUSE AMYLOID DEPOSITS IN THE PRESUBICULAR PARVOPYRAMIDAL LAYER IN ALZHEIMER'S DISEASE. S. Itagaki*, H. Akiyama*, H. Tago*, P.L. McGeer and E.G. McGeer. (SPON: S.C. Sung). Kinsmen Laboratory, Univ. British Columbia, 2255 Wesbrook Mall, Vancouver, B.C. V6T 1W5, Canada

The presubiculum is a significant destination for fiber projections from anterior thalamic nuclei. In turn, fibers from this area project to the entorhinal cortex to close the limbic circuit originally described by Papez. Immunostaining for β-amyloid protein or modified Bielschowsky's silver staining has revealed the occurrence of diffuse amyloid deposits in the presubiculum in all Alzheimer cases so far examined (n=14). Observations on serial blocks showed these deposits to be localized in the presubicular parvopyramidal layer of the presubiculum proper, the transsubiculum and the subiculum, while the parasubiculum was devoid of such deposits. Bielschowsky's staining indicated that the amyloid deposits preceded the appearance of neurofibrillary tangles and neuropil threads in this region. The aggregated microglial reaction which was characteristically associated with the classic senile plaques was absent. Since the presubiculum also receives afferents from frontal and parietal association cortices, the changes in this area in Alzheimer's disease could contribute to isolation of the hippocampal formation from other cortical areas. Furthermore, convergence of cortical inputs might play a significant role in the formation of diffuse amyloid deposits in the presubiculum.

414.38

QUANTITATION OF CELL NUMBER AND SIZE IN AMYGDALA IN ALZHEIMER'S DISEASE. S.A. Scott, C.Knox,* S.T. DeKosky,and Scheff, S.W. Depts. of Anat. & Neurobiol. and Neurology, Lexington VA and Univ. Kentucky Med. Ctrs. and Sanders-Brown Center on Aging, Lexington, KY 40536.

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

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

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

414.39

APPEARANCE OF DIVERSE BRAIN-REACTIVE ANTIBODIES IN SERA OF AGING NZB/BINJ MICE. V. Apte*, E.N. Ahanotu*, H. Lal, and M.J. Forster.(SPON: I. Korr). Department of Pharmacology, Texas College of Osteopathic Medicine, Fort Worth, TX 76107-2690.

The appearance of brain-reactive antibodies (BRA) and behavioral impairments occur at relatively young ages in autoimmune-prone NZB/BINJ mice when compared with C57BL/6NNia mice, a non-autoimmune strain (Lal & Forster, *Neurobiol. Aging*, 9:733, 1988). The purpose of this experiment was to characterize the brain cell membrane antigens recognized by BRA of NZB/BINJ mice, for comparison with BRA of aged C57BL/6NNia mice (Ahanotu et al., *FASEB J.*, 3:A571). Cell membrane proteins were extracted from dissociated cells obtained from whole brains of young ICR mice using N-lauroyl sarcosinate. The extracts were then separated by polyacrylamide gel electrophoresis and immunoblots were performed with these antigens using serum from NZB/BINJ mice aged 2, 10, or 16 months (ns=15). While serum from all age groups recognized an 18 kD species, the 10- and 16-month-old groups recognized a variety of other antigens (including 21, 24, 35, 36, 43, and 47-kD species). These findings suggest that the appearance of age-related behavioral deficits in NZB/BINJ mice may be related to the appearance of one or more specific serum BRA populations.[Supported by NIH grants AG06182 (MJF), AG07695 (HL), and NIH-BRSG grant RR05879].

414.40

CULTURES OF HUMAN BRAIN CELLS FROM AUTOPSIES OF ALZHEIMER'S, PARKINSON'S AND CONTROL PATIENTS. U. Rovigatti, M. Notohanmiprodjo*, and P. Riely. Institute for Biogerontology Research, Sun City, AZ 85351.

Although a well-known dogma of neuroscience holds that adult brain cells are post-mitotic and therefore not capable of proliferating, we have attempted to maintain in culture cells from different regions of brain autopsies obtained from Alzheimer's, Parkinson's or normal patients. Different culture conditions have been evaluated and optimized for approximately fifteen different regions from brain autopsies on 12 Alzheimer's cases, 4 Parkinson's cases, and 4 control cases. Under optimal conditions, a small percentage of brain cells (.01-.001%) can be maintained in vitro in viable condition for variable periods of time. Although we are still investigating the nature of these cells, their neuronal or at least non-glial nature is suggested by the fact that 1) glial cells do not appear to attach and proliferate under our culture conditions; 2) cultured brain cells do not stain with endothelial or glial specific antibodies (e.g., Factor VIII, GFAP); 3) cultured brain cells are stained by the anti-neurofilament antibodies SMI-32 and SMI-33 and also, in a smaller percentage of cells, with antibodies against phosphorylated neurofilaments. Surprisingly, some of these brain-derived cells appear to divide under our culture conditions, as shown by increase of cell numbers and thymidine incorporation. We conclude that cells from adult brains can be maintained in culture for longer times than previously thought. Although further studies are required for their characterization, such cultured cells may provide an interesting model for studies of CNS cells in both normal and pathological conditions.

CIRCUITRY AND PATTERN GENERATION: VERTEBRATES

415.1

COSTS AND BENEFITS OF HIGH FIRING RATE SENSITIVITY IN NEURONS **J. Elek*, M. Hulliger*, A. Prochazka, R.S. Smith & S. Vincent*. Division of Neuroscience, University of Alberta, Edmonton, Alberta, T6G 2S2, CANADA**

Most mammalian neurons transmit time-varying signals in the form of rate-modulated trains of action potentials. If the depth of modulation in response to a given input increases, this represents an increase in gain. Increased resolution might also be expected, but the variability of inter-spike intervals generally increases along with gain. Is the net result an increase or a decrease in resolution? On the costs side, energy is required for axonal and synaptic transmission. Transmitter utilisation increases: depletion may exceed supply, leading to synaptic failure. We have examined these issues in ensembles of muscle spindle afferents, whose gain is known to be deeply modulated by fusimotor action from the CNS, according to behavioural context. Stretch responses of over 200 cat Ia afferents obtained in acute experiments with and without fusimotor action were summated in groups of varying size. The resulting ensemble pulse trains were used to drive a simple low-pass filter model of a neuronal membrane. We conclude that:

1) at the highest firing rates energy utilisation more than doubles over basal values; pacemaker and/or transmission failure may set in after 20-30s.
2) transmitter depletion may lead to synaptic failure even earlier.
3) increased variability of inter-spike intervals in individual afferents only erodes resolution in the decoded output of very small ensembles (<5).
4) thus increased gain and resolution are the benefits, the risk of transmission failure is the cost: this may explain why gains are not kept high permanently.

Supported by Alberta Heritage Foundation and Medical Research Council.

415.2

DIFFERENTIAL MODULATION OF DOUBLE BURSTING MUSCLES IN THE CHICK. R. M. Johnston and A. Bekoff. Dept. of EPO Biology, University of Colorado, Boulder, CO 80309

Many behavioral studies have examined the role of sensory feedback in shaping the typical features of the underlying motor patterns. It has been shown for cat locomotor behaviors that afferent input influences the activity of one burst of a double bursting knee muscle while not affecting the activity of the other burst. We are interested in determining if individual bursts of double bursting muscles in the chick show unique modulation by afferent feedback.

In this study, we examined EMG recordings obtained from intact chicks during walking, swimming and airstepping. These behaviors are similar in that they are locomotor behaviors involving alternating limb movements, but with clear differences in afferent feedback. Specifically, we examined two double bursting knee muscles: femorotibialis (FT) and iliofibularis (IF). FT is a uniarticulate knee extensor. IF is a biarticulate muscle which is involved in hip extension and knee flexion. To examine the effects of afferent input, muscle synergies and the association between individual burst durations of FT or IF and cycle periods were compared among the three behaviors.

Our results suggest that the motor output of the individual bursts of these two double bursting muscles is differentially regulated by afferent input. In addition, we found that modulation by afferent feedback changes the synergies for the uniarticulate knee muscle, FT, but does not change the synergies for the biarticulate knee muscle, IF.

Supported by NIH grant NS20310.

415.3

FORCE/FREQUENCY RELATIONS IN SINGLE HUMAN THENAR MOTOR UNITS. B. Bigland-Ritchie, C. K. Thomas and R. S. Johansson. John B. Pierce Fndn, New Haven, CT. & Physiology Dept, Umeå University, Sweden.

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

Conventional sigmoid force/frequency curves were generated for all units, with minimal twitch fusion evident at 5-8 Hz, 50% maximum force below 15 Hz, and maximum force between 50-100 Hz. The rates giving 50% maximum force were influenced by the sequence of stimulus pattern delivery and by the unit's previous activation history, but were not well correlated with twitch contraction or 1/2 relaxation times. When stimulating at 1-50 Hz EMG potentials remained constant throughout each pulse train; but during 100 Hz stimulation their amplitudes declined rapidly, despite a well maintained force plateau.

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

415.4

STIMULUS PATTERNS FOR FORCE GENERATION BY SINGLE HUMAN THENAR MOTOR UNITS. C. K. Thomas, R. S. Johansson and B. Bigland-Ritchie. John. B. Pierce Fndn, New Haven, CT. & Physiology Dept, Umeå Univ, Sweden.

The minimum excitation rates required for optimum force generation were investigated in 21 single human motor units of thenar muscles by stimulating individual motor axons of the median nerve above the elbow as described previously (Westling et al, Neurosci Abst. 14, 1988). First, two pulses were delivered 500 ms apart; then repeatedly at intervals reduced to 5ms in increments of 20-5 ms. The interval generating maximum twitch summation was then repeated as a third pulse was again delivered at progressively shorter intervals. This process was repeated until 6 such intervals were established; a procedure similar to that used for cat motor units by Burke et al (Brain Res. 109: 515,1976). Most human units generated maximum force in pulse trains where the first 2 shocks were delivered 5-10 ms apart, followed by longer intervals. These rates were compared to those seen in strong human voluntary contractions.

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

415.5

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

It is well known that mammalian enteric ganglia possess an integrative system for rhythmic discharge analogous to that of the vertebrate central nervous system. Sympathetic ganglia, on the other hand, have been traditionally considered as simple relay mechanisms with no such integrative function. However, observations made in isolated superior cervical ganglia (SCG) of rabbits and rats suggest that this ganglion can, under certain conditions, fire ongoing spontaneous rhythmic discharge as single spikes or bursts of spikes occurring at regular intervals. Ganglia were placed in oxygenated Locke's solution with its rostral end aspirated into a capillary suction electrode. Rhythmic discharge appeared on treatment with 4AP, emetine or Cs^+. In the majority of rabbit SCG intense, often rhythmic, discharge was observed in untreated ganglia. This discharge subsided in 10-60 min, but disengagement from the electrode and resuctioning caused reappearance of the discharge. This indicates that suction pressure may have caused sustained transmitter release which triggered the rhythmic firing. Chronic pretreatment of rats with 6-hydroxydopamine or reserpine did not interfere with the ability of drugs to induced rhythmic firing in the SCG. The results suggest that putative catecholaminergic SIF "interneurons" are not involved in the generation of rhythm and that sustained transmitter release by any procedure could trigger the rhythmic discharge. (Supported in part by NIH-MHRDP Grant HL-7434-10.)

415.6

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

The objective of this study was to characterize the motor pattern generating capability of spinal cord-hind-limb explants taken from Balb/C mice aged birth to 4 days. Spontaneous and NMDA evoked EMG activity from the gastrocnemius (G) and tibialis anterior (TA) muscles was recorded and bursting sequences exhibiting the same timing as intact locomotion were included for analysis. Spontaneous locomotor rhythm occurred in only 4 of 76 experiments and those 4 cases involved explants $\leq$ 2 days of age. Separately, explants were tested with and without: 1) hemisection of the spinal cord; and 2) magnesium (1.0 mM) present in the perfusion solution. Of all combinations, nonhemisected explants perfused with magnesium free artificial CSF were most responsive to bath application of 1 - 5 µM NMDA. Using that preparation, motor rhythm was most often observed in 2 day old mice. The threshold dosage for evoking rhythm tended to be lower (1.5 - 2.0 µM) for animals $\leq$ 2 days of age. The EMG pattern for G and TA activity included sequences of: 1) synchronous bursting; 2) mixed synchrony and alternation; and/or 3) irregular alternation. The alternating activity observed in vitro was often an alternation of entire bursting sequences rather than a cycle-to-cycle phasing. (NIH Grant # 1 R29 NS 25250-01.)

415.7

VERTEBRATE RHYTHM GENERATION WITHOUT RECIPROCAL INHIBITION: SENSORY INTERNEURONES NOT INVOLVED. K.T. Sillar* & S.R. Soffe. (SPON: W.J. Heitler) *Gatty Marine Lab., Univ. St. Andrews & Dept. Zoology, Univ. Bristol, UK.

In Xenopus embryos, a role for glycinergic inhibition during swimming can be demonstrated by application of the glycine antagonist, strychnine. Rhythm generation that persists in the presence of strychnine depends critically on the activation of excitatory amino acid receptors, being abolished by 30 uM APV or 1mM kynurenic acid. While the normal excitatory drive for swimming from descending excitatory interneurones should be unaffected by strychnine application, it remained possible that activity in sensory interneurones (normally inhibited during swimming) is released by strychnine application and could contribute to rhythm generation.

We have therefore recorded intracellularly from sensory interneurones in the presence of strychnine. The interneurones discharge impulse bursts at the onset of rhythmic activity but are inactive thereafter. We conclude that they are not directly involved in rhythm generation, re-emphasising a central role for descending excitatory interneuronees.

415.8

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

An *in vitro* preparation of the isolated brainstem-spinal cord of the neonatal rat is presently being used in our laboratory to study cellular and synaptic mechanisms underlying the neurogenesis of respiratory movements in mammals. Previous studies utilizing this preparation have suggested that neuron populations in the ventrolateral reticular formation of the medulla are important for respiratory rhythmogenesis and motor pattern formation (Smith & Feldman, Soc. Neurosci. Abs.14:1060, 1988). We have now constructed detailed maps of the spatiotemporal patterns of neuronal activity in these areas. Extracellular recordings of neurons in the vicinity of nucleus ambiguus from the level of the caudal facial nucleus to areas caudal to the obex were made with dye-filled glass microelectrodes (2% pontamine sky blue in 0.5 M NaAc solution; impedance=7-12 MΩ). We identified and mapped five major respiratory cell types with distinct temporal discharge patterns: inspiratory (I), pre-I [discharge starting in late expiratory (E) phase and continuing into I phase], tonic E, late E, and biphasic E cells [discharge bracketing I phase]. Each of these cell types was found throughout the ventrolateral reticular formation with a relatively high concentration of tonic and late E phase neurons at the level of rostral nucleus ambiguus. This distribution resembles the spatial distribution of respiratory neurons in the ventral respiratory group of the medulla *in vivo*. These results demonstrate the presence of a complex spatiotemporal pattern of neuronal activity in the ventrolateral medullary reticular formation of the *in vitro* preparation. Neuroanatomical tract tracing techniques are currently being used to map the distribution of cranial motoneurons, bulbospinal and propriobulbar respiratory cells in these regions for comparison to the map of respiratory neuron activity. These studies provide the necessary groundwork for further analyses of the neuronal mechanisms underlying respiratory rhythm and pattern generation in the *in vitro* system. Supported by NIH Grants HL 40959 and HL 02204.

415.9

LOCATION OF TRIGEMINAL PRE-MOTONEURONS AND THEIR RELATIONSHIP TO GLUTAMATERGIC CELLS IN THE GUINEA PIG BRAINSTEM. J.E.Turman, Jr*., S.H. Chandler and R.S. Fisher. Dept. of Kinesiology and Psychiatry and the Brain Research Institute, UCLA, L.A., CA. 90024.

We initiated a series of anatomical studies to elucidate the neuronal circuitry underlying the generation of rhythmic jaw movements. Initial experiments using iontophoretic injections of WGA-HRP in the trigeminal motor nucleus identified trigeminal pre-motoneurons (Trig-PMn). Trig-PMn were found in the mesencephalic nucleus of V (Mes 5) ipsilaterally, principal sensory nucleus of V(Pr 5) bilaterally, spinal trigeminal nucleus oralis and interpolaris (Sp5O,Sp5I) bilaterally, intertrigeminal region ipsilaterally, and bilaterally in the parvocellular reticular formation extending from the hypoglossal nucleus to the caudal aspect of the trigeminal motor nucleus. No labeling was found in the large cells of the gigantocellular reticular nuclei. Previous physiological experiments from our laboratory have impicated an excitatory amino acid as a neurotransmitter that mediates reflex and cortically induced jaw movements. Therefore, we performed a series of glutamate mapping studies to determine which brainstem regions involved in jaw movements were also glutamatergic. Following a transcardial perfusion of 5% carboiimide and 5% glutaraldehyde, sections of the brainstem region were incubated for 24 hours in a monoclonal antibody directed against glutamate. The ABC immunohistochemical process was utilized. Glutamatergic cells were found in the inferior olive, solitary nucleus, external cuneate nucleus, prepositus hypoglossal nucleus, Sp5I, Sp5O, vestibular nuclei, ventral cochlear nucleus, superior olive, Mes.5, parabrachial nuclei, central nucleus of the inferior colliculus and portions of the reticular formation including nucleus gigantocellularis, paragigantocellularis and parvocellularis. Currently we are performing a double labeling study combining the retrograde tracer, gold bound WGA-HRP, with glutamate immunohistochemistry to determine the locations of specific glutamatergic trigeminal pre-motoneurons. Funded by NIH-NIDR grants DE 07618 and DE 06193.

415.10

A DESCRIPTION OF THE PROPERTIES OF TRIGEMINAL COMMISSURAL INTERNEURONS IN THE RABBIT. R. DONGA and J.P. LUND. Cent. Res. Sci. Neurol. Univ. de Montréal, Montréal, CANADA, H3C 3J7.

The physiological identification of trigeminal interneurons by antidromic stimulation of their terminals is usually difficult because of short conduction distances. One is left with the option of classifying interneurons by excluding first order afferents, motoneurons and lemniscal neurons. In the work to be described, we were able to identify groups of interneurons terminating in the contralateral fifth nerve motor nucleus (Mot. V) which were located in the intertrigeminal area and the most anterior portion of subnucleus oralis (Landgren et. al. Exp. Br. Res., 65: 98, 1986). It was possible to stimulate the Mot. V and record antidromic spikes on the contralateral side. Using standard criteria for antidromic identification, 37 neurons were identified as commissural interneurons. Their latency range was 0.4 to 2.0 ms and conduction velocities 3.5 to 17.5 M/s. 14 units had intra-oral, 4 had peri-oral, 2 had both intra- and peri-oral receptive fields. Peripheral receptive fields for the rest of the units were not determined. In addition, many of these neurons were excited at short latency (<3.5 ms) by stimulation of the contralateral sensorimotor cortex. The discharge pattern of eight of these units was phasically modulated during fictive mastication. The role of these neurons in the bilateral co-ordination of mastication and the jaw opening reflex is under investigation. Supported by the Canadian MRC.

415.11

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

The hypothesis that central control for the oesophageal peristalsis is intrinsically bilateral was investigated. In rats anaesthetized with urethane, deglutitive and cholinoceptor-mediated oesophageal peristalsis were produced by application of bicuculline methiodide and muscarine, respectively, to the surface of the nucleus of solitary tract (NTS; 50-100 pmol, 0.1 μl). **1.** Topical pretreatment with methscopolamine (5-10 pmol, 0.1 μl) selectively blocked the ipsi- but not contralaterally-evoked peristalsis. **2.** Ipsilateral acute or subchronic cervical vagotomy resulted in failure of distal and decrease in cervical oesophageal responses; supranodosal section of the IXth and Xth nerves abolished oesophageal, but not pharyngeal, activity evoked from ipsilateral NTS with a slight reduction of oesophageal response amplitude evoked from the contralateral NTS. **3.** Microinjections of the non-selective glutamate antagonist, gamma-D-glutamyl-glycine or the NMDA antagonist, D,L-2-amino-7-phosphono-heptanoic acid (25-50 pmol, 0.5-1 nl) into ipsilateral nucleus ambiguus (AMB) markedly inhibited the evoked oesophageal responses, while no effect was observed after microinjections of these drugs into the contralateral AMB. Our results are inconsistent with an obligatory bilaterality of central oesophageal motor control. Instead, they suggest that deglutitive projections from the NTS oesophageal premotoneurons to the AMB oesophageal motoneurons are predominantly ipsilateral and that glutamate or aspartate may serve as excitatory neurotransmitter in this pathway.

415.12

DARWINIAN VIEW OF LANGUAGE: A WELL-FORMED SENTENCE AS ANALOGOUS TO SPECIATION AND THE IMMUNE RESPONSE.
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A possible preadaptation for the neural machinery underlying language and thought can be seen in that required for planning sequential movements. If it isn't a standardized movement sequence such as the basketball free-throw, one has to generate a family of variations on an approximate string of commands, and then judge each of those candidate strings against memories of what strings succeeded in the past for similar-but-not-identical conditions. Thus one needs an array of planning tracks rather like a railroad marshaling yard: a *massively-serial* architecture. **Consider the transition between such variations-on-a-theme and the nearly-identical strings of a "Choral Mode."** The easy way is for the most successful strings (the ones scoring highest in comparison to memories) to reproduce, replacing low-ranked strings -- but perhaps with some copying errors. Some daughter strings may be even better than their parent string, have an even better fit to emerging memories of past successes and failures. As this shaping is repeated during "get set," a near-uniform choral-like population of strings may emerge. I have called this a Darwin Machine (*Nature* 330:33-34, 1987).

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

CIRCUITRY AND PATTERN GENERATION: INVERTEBRATES AND MODELS

416.1

A 448-CHANNEL OPTICAL MONITORING OF NEURAL SIGNALS FROM APLYSIA GANGLION. M. Nakashima*, S. Yamada*, S. Shiono* and M. Maeda*. (SPON: O. Koizumi). Central Research Laboratory, Mitsubishi Electric Corp., Amagasaki, Hyogo 661, Japan.

The multi-channel optical recording method has an advantage to detect neural signals simultaneously from a large number of neurons. The 128-channel optical recording method was applied to observe neural activities from the abdominal ganglion during gill withdrawal reflex elicited by mechanical stimulation to siphon (Wu, J.-Y. et al., Experientia, 44:369, 1988).

To improve the spatial resolution of the optical method and thus to detect more active neurons, we made a 448-channel optical monitoring apparatus, using a round-shaped 448 photodiode array (Hamamatsu Photonics, Hamamatsu, Japan). One photodiode array element is 0.9x0.9 mm, with 0.23 mm wide isolation region and hence the active photodiode element size is 0.67x0.67 mm. An electrical output from each photodiode element was amplified, digitized through multiplexer (1 kHz/ channel) and stored in a magnetic disk.

The 448-channel optical apparatus was applied to the semi-intact abdominal ganglion preparation with siphon and gill remained. Action potential activites from the ganglion were observed. Some of the experimental results obtained will be presented.

416.2

CALCIUM CURRENTS AND SYNAPTIC TRANSMISSION IN HEART INTERNEURONS OF THE MEDICINAL LEECH. J.D. Angstadt and R.L. Calabrese. Dept. of Biology, Emory University, Atlanta, GA 30322.

Interneurons controlling heartbeat in the leech (HN cells) produce regenerative Ca-dependent plateau potentials with thresholds near -55 mV (Arbas and Calabrese, J. Neurosci. 7:3945-3952, 1987). We have investigated the ionic currents underlying these potentials using the single-electrode voltage clamp technique. All experiments were performed in 0 mM Na saline (Na replaced with N-methyl-D-glucamine). From a holding potential of -60 to -70 mV, inward currents are elicited by depolarizing voltage steps beginning at approximately -55 mV. Inward currents persist when Ca is replaced with Ba or Sr and are increased in amplitude when the extracellular Ca concentration is raised. In contrast, inward currents are eliminated when Ca is replaced with Co, Mn, or Ni. These data are consistent with the presence of a voltage-dependent calcium current in HN cells.

Each bilateral pair of HN cells in ganglia 3 and 4 are connected by reciprocal inhibitory synapses. We examined synaptic transmission between HN cell pairs in isolated ganglia 3 and 4 in the absence of spikes (0 mM Na saline). The presynaptic cell was voltage clamped at a holding potential of -50 to -60 mV and stepped to either -50 or -40 mV for 3 seconds (these voltage steps mimic the known voltage excursions of HN cells during normal rhythmic activity). The contralateral HN cell responded with a prolonged IPSP exhibiting a peak-plateau waveform. We are now attempting to relate these synaptic responses to the above calcium currents and assess their role in the generation of normal rhythmic activity.

416.3

KINETIC ANALYSIS OF MEMBRANE CURRENTS IN THE HEART INTERNEURONS OF THE MEDICINAL LEECH. T.W. Simon, J.D. Angstadt & R.L. Calabrese. Dept. of Biology, 1555 Pierce Drive, Emory University, Atlanta, GA 30322

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

We have developed this method of analysis for three voltage dependent conductances by using measured τ's and steady state values over a range of clamp potentials to calculate rate constants for state transitions of these conductances. These rate constants are exponential functions of membrane potential (Hodgkin & Huxley, 1952, J.Physiol. 117:500-544).

We were able to model I_h, a hyperpolarization-activated inward current, which contibutes to recovery from inhibition (Angstadt & Calabrese, 1989, J. Neurosci. in press) and successfully simulated its behavior in current- and voltage-clamp experiments. Whole cell recording of an I_{K^+} resembling the delayed rectifier were also successfully simulated. We modeled an $I_{Ca^{2+}}$ with a rapid (τ = 10-20ms) and a slow component (τ = 100-300ms).

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

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

416.4

QUANTITATIVE ANALYSIS OF NON-SPIKING SYNAPTIC TRANSMISSION BETWEEN SPIKING STOMATOGASTRIC NEURONS. K. Graubard, J.A. Raper* and D.K. Hartline, Dept. of Zoology, U. of Wash., Seattle, WA 98195; Max Planck Inst. Enwicklungsbiologie, Tubingen, W.Germany; U. of Hawaii, Honolulu, HI 96822

Spiking neurons of the pyloric system in the spiny lobster stomatogastric ganglion release inhibitory transmitter as a graded function of presynaptic membrane potential at voltages subthreshold for spiking (Graubard et al. *J. Neurophysiol.* 50:508,1983). The following characteristics in TTX were established when current was injected into the presynaptic soma and the inhibitory response was monitored in the postsynaptic soma:

1. In response to sinusoidal injected current the postsynaptic voltage showed oscillations with a broad peak in amplitude centered around 0.5 Hz, corresponding approximately to the natural frequency of the network. High frequency gain roll-off ca. 4 dB/octave; corner frequency ca. 1-2 Hz.
2. Current pulses, 2.5 secs in duration, were used to generate input-output curves. The postsynaptic soma voltage was a steeply increasing function of presynaptic soma voltage (tested up to 20 mV above TTX rest). For presynaptic PD cells, the relationship could be fitted well with an exponential having an e-fold increase in peak postsynaptic response for 11 mV of peak presynaptic depolarization (range 7-20 mV; n=7). The corresponding value for the maintained response was several mV larger.
3. Ca. 5 nA depolarizing or hyperpolarizing current pulses injected into the presynaptic soma just before a test pulse altered the input-output relationship. The X-axis intercept (postsynaptic response=0) shifted an average of 5 mV more positive for depolarizing conditioning pulses and 6 mV more negative for hyperpolarizing ones. Typically, the exponent was not much changed (less than 2 mV, on average, for presynaptic PD cells).

Supported by NIH grants NS15697 (K.G.) and NS15314 (D.H.)

416.5

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

In the crab, Cancer borealis, the gastric rhythm of the stomatogastric ganglion (STG) is rarely spontaneously active in control saline, even when the STG is left attached to neural inputs from the anterior Commissural (CoG) and Oesophageal (OG) Ganglia. We have been applying a variety of putative neuromodulators, known to be present in inputs to the crab STG, in hopes of finding a substance or substances that will activate the gastric rhythm. We now find that the extended FMRFamide-like peptide SDRNFLRFamide, originally purified from Homarus americanus (Trimmer et al., J. Comp. Neurol. 266: 16-26, 1987) either applied alone (10^{-8}M to 10^{-7}M) or in conjunction with the muscarinic agonist, pilocarpine (10^{-8}M to 10^{-4}M), reliably initiates robust gastric activity. Both pilocarpine and SDRNFLRFamide also strongly activate the pyloric rhythm. Since many of the neurons of the STG can fire in both pyloric or gastric time, these substances allow us to explore the mechansims by which neurons are shifted from one motor pattern to another by modulators. Supported by NS17813.

416.6

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

The branchiostegite nerves (BN) originate from the dorsal-lateral surface of the thoracic ganglion posterior to the scaphognathite nerves and innervate the walls of the branchial chambers.

The sensory components include mechanoreceptors and other as yet unidentified receptors. Stimulation of the BN modifies the ventilatory rhythm in several ways including changes in pumping rate, switch from forward to reversed pumping and cause pausing.

The five motoneurons in each BN innervate a band of anterior branchial enlarger muscles (ABEM). These muscles run from the dorsal carapace to the flexible dorsal roof of the branchial chamber, they receive bursts of spikes in phase with scaphognathite induced branchial pressure waves and their spike frequency is proportional to ventilation rate. The ABEM motoneurons are silent during ventilatory reversals when branchial pressure becomes positive. Artificially raising branchial pressure silences, while lowering the pressure increases ABEM spike frequency. Thus, these motoneurons demonstrate both programed and reflexive patterns of activity. Contraction of the ABEMs will facilitate the movement of water through the gills by expanding the roof of the gill chamber in a diaphragm pump-like manner. (N.S.E.R.C. Canada)

416.7

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

In vivo, the giant pedal dopaminergic interneuron (R.Pe.D1) of the pond snail Lymnaea stagnalis makes monosynaptic connections with its follower cells in the parietal and visceral ganglia. These follower cells of R.Pe.D1 in Lymnaea are identified and their connections are well characterized. In order to test the appropriateness of these connections in vitro, the R.Pe.D1 and its follower cells were cultured in Lymnaea conditioned medium. Lymnaea R.Pe.D1 was found to make appropriate chemical connections with its follower cells. In related snail Helisoma trivolvis, the neuron homologous to Lymnaea R.Pe.D1 is found in the left pedal ganglion (L.Pe.D1). The follower cells of Helisoma L.Pe.D1 have been located but their connections have yet to be characterized. The R.Pe.D1 from Lymnaea was plated with the follower cells of L.Pe.D1 from Helisoma in Lymnaea/Helisoma conditioned medium. All these cell types exhibit extensive neurite outgrowth within 18-24 hr of plating. Electrophysiologically, R.Pe.D1 from Lymnaea makes either excitatory, inhibitory or biphasic connection only with the prospective follower cells from Helisoma. These studies demonstrate the ability of homologous cells from two different species to establish chemical connections, which appear specific.

416.8

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

An interaction between inhibitory and excitatory rhythm generators has been shown to produce the mammalian respiratory rhythm, but little is known of the circuitry within these CPGs. The intraneuronal circuitry underlying respiratory behaviour in the pond snail Lymnaea has already been described. The interneurons IP3 and VD4 have reciprocal inhibitory connections with each other and are implicated in the opening (expiration) and closure (inspiration) movements of the pneumostome. Interneuron R.Pe.D1 can initiate the respiratory cycle by post-inhibitory rebound excitation (PIR) of the IP3 neuron, which in turn excites VD4 by PIR and the cycle repeats. In order to test the sufficiency, necessity, adequacy and modulatory role of these neurons in the CPG, these identified neurons were cultured in vitro. All these cell types exhibit extensive neurite outgrowth within 18-24 hrs of plating in conditioned medium. When all three cell types were plated together in the same dish, it was possible to initiate the respiratory cycle by the excitation of R.Pe.D1 and the network became coupled, IP3 and VD4 interneurons bursting alternatively. This mimics the situation seen during in vivo active respiratory behavior. These studies therefore suggest that at least a relatively simple CPG can be reconstructed in culture and its activities can be studied and manipulated in greater detail.

416.9

GABAERGIC MODULATION OF THE RESPIRATORY AND LOCOMOTORY CENTRAL PATTERN GENERATORS (CPG) OF LYMNAEA. J.E. Richmond, N.I. Syed, K. Lukowiak and A.G.M. Bulloch. Dept of Physiology, University of Calgary, Calgary, Alta. Canada T2N 4N1.

The pedal ganglia of Helisoma trivolvis contain a network of GABA-immunoreactive neurons. To date none of the Helisoma pedal neurons have been characterized. However, in the related basommatophoran pulmonate, Lymnaea stagnalis, the involvement of pedal neurons in respiratory and locomotory activity has been recently demonstrated. The circuitry underlying these behaviors has been partially characterized and therefore provides an opportunity to investigate the role of GABA in modulating the patterned activity generated in the pedal ganglia. This was tested by bath application of GABA while recording intracellular activity from pedal and visceral neurons known to be involved in the respiratory and locomotory rhythms. GABA caused an inhibition of spontaneous respiratory cycles. In addition, cycles of the respiratory rhythm stimulated by depolarization of the right pedal neuron 1 (R.Pe.D1) were abolished in the presence of GABA. GABA induced hyperpolarizations of both the right and left Pe.D1 neurons. Neurons of the pedal A and D clusters, which are part of the locomotory CPG, were also hyperpolarized by GABA. These preliminary data suggest that GABA has inhibitory effects on both respiratory and locomotory CPG neurons.

416.10

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

An important problem in the production of motor patterns is the interaction between central pattern generators (CPG) and other components of the nervous system. We are currently investigating this, using oviposition in the locust (*Locusta migratoria*) as a model system. Oviposition digging is a relatively stereotyped behaviour, produced at least in part by a CPG located in the VIIIth (terminal) abdominal ganglion (K.J. Thompson, *J. Exp. Biol.* 122: 387, 1986). The digging rhythm is produced by isolated ganglia taken from females interrupted during oviposition, but it generally ceases within 30 min. It can be restarted by electrically stimulating peripheral nerves that project from the ovipositor to the CNS. These nerves contain sensory axons from the ovipositor, but do not appear to contain any motor axons. The initiated rhythm outlasts the stimulation by several (often tens) minutes, and the process can be repeated several times. This suggests that sensory input may be important in either the initiation or maintenance of this centrally-generated rhythm.

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

416.11

INTERSEGMENTAL COORDINATION IN THE LOCOMOTOR SPINAL CPG: THEORY AND EXPERIMENT. T.L.Williams, K.A.Sigvardt, N.Kopell* & G.B.Ermentrout*. SGHMS (U.London), UC-Davis, Boston U. & U.Pittsburgh.

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

Supported by SERC, NINCDS, NSF, AFOSR.

416.12

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

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

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

416.13

CONVERGING AND DIVERGING CONNECTIONS IN NEURAL NETWORKS: HOLOGRAPHY AND COMPUTATIONAL POTENTIAL OF THRESHOLDS AND SYNAPTIC WEIGHTS. G. J. Mpitsos and R. M. Burton. Hatfield Marine Science Center, Newport, OR 97365, and Department of Mathematics, Oregon State University, Corvallis, OR 97334.

Findings of chaos and variability in buccal-oral motor patterns of our experimental animal, the sea slug Pleurobranchaea, motivated us to examine the ability of neural networks to learn to transmit analog chaotic signals (Mpitsos, G. J., Burton, R. M., and Creech, H. C., Brain Res. Bull., 21:539, 1988). We show here that simple connectionist networks can also learn to perform a variety of computations on chaotic signals, and, using different input pathways, that they can learn several different tasks at the same time. Trainable synaptic weights were sufficient for networks to learn many tasks, but by including trainable thresholds the range of tasks was greatly increased, suggesting that examination of trainable neuronal thresholds in biological systems may prove fruitful, as has been examined behaviorally (e.g., Mpitsos, G.J., and Cohan, C. S., J. Neurobiol. 17:499, 1986). When convergence is spatially separated from divergence, as in networks containing one input unit, one output unit, and only one layer of hidden units ("interneurons"), memory is distributed unevenly throughout the network. But when convergence and divergence are mixed, as between two hidden layers, memory is more holographically or evenly distributed. Although training set all synaptic weights to optimal levels, networks naturally generated many "lazy" synapses which had little effect when removed from network computations. Lazy synapses may be a natural product of converging/diverging biological networks, and epiphenomenally may find adaptive use in generating variable, shifting motor patterns. Thus, while connectionist networks are not biological they may be helpful for identifying simple, universally applicable principles, and, thereby, may provide insight into the types of phenomena to examine in biological systems. Supported by AFOSR 89-0262.

416.14

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

We have used neuroethological and neurobiological data to design model neural networks that can generate complex, adaptive, and autonomous behavior. We have devised a simulated insect and several controllers using neuron-like elements. The pattern of interconnections of a controller for hexapod locomotion was guided by those that have been described experimentally by Pearson (1976). This controller generates gaits similar to those described by Wilson (1966) in insects, and is remarkably robust to lesions of different elements. We have also constructed a controller for feeding behavior, using the data of Gelperin et al. (1971) and Weiss et al. (1986). The model insect can sense and follow odor gradients, and shows aspects of food arousal and satiation. In addition, the model insect can appropriately integrate different behaviors. For example, if it encounters an obstacle while following an odor gradient, it can follow around the edge of the obstacle, and then continue following the odor gradient. We intend to make the model more detailed and realistic so that it can be used to generate experimentally testable hypotheses about the neurobiology of insects. We also intend to use these ideas for the design of artificial devices capable of adaptive, autonomous behavior.

416.15

SIMULATION OF THE EXPERIMENTALLY ESTABLISHED SEGMENTAL, SUPRASPINAL AND SENSORY CIRCUITRY UNDERLYING LOCOMOTION IN LAMPREY. A. Lansner*, Ö. Ekeberg*, H. Tråvén*, L. Brodin*, P. Wallén*, M. Stensmo* and S. Grillner. Nobel Inst. for Neurophysiology, Karolinska Institutet, and Dept. of Numerical Analysis and Computing Science, Royal Institute of Technology, Stockholm, Sweden.

The lamprey nervous system in vitro serves as a model for the cellular basis of vertebrate locomotion. Briefly the segmental premotor interneurons which activate motoneurons during fictive locomotion are rhythmically active and their interactions can explain the segmental burst generation (Buchanan & Grillner, 1987; Grillner et al., 1988). This network is subject to a powerful sensory entrainment by intraspinal stretch receptor neurons (Grillner et al., 1981; 1984), which provide ipsilateral monosynaptic EPSPs on the network. The reticulospinal initiation is exerted via monosynaptic EPSPs in all network interneurons (Ohta & Grillner, 1989). Efference copy information from the spinal level modulates the reticulospinal discharge (Dubuc & Grillner, 1989). Realistic simulations of this entire circuitry has been made by modelling each type of neuron with relevant types of membrane channels (Na^+,K^+,K^+_{Ca},Ca^{2+}, conventional EPSP and IPSP channels, and voltage dependent NMDA channels). The latter are of particular importance during slow swimming (Brodin & Grillner, 1985, 1986; Sigvardt et al., 1985; Wallén & Grillner, 1987). The cells have soma and three dendritic compartments. The network simulates a large variety of experimentally established findings.

416.16

COMPUTER SIMULATION OF OSCILLATION IN MUTUALLY INHIBITORY HEART INTERNEURONS IN LEECH.

[1] E. De Schutter*, [2] T.W. Simon, [2] J.D. Angstadt and [2] R.L. Calabrese (SPON: P. Lennard). [1] Dept. of Neurology, University of Antwerp, Belgium; [2] Dept. of Biology, Emory University, Atlanta, GA 30322.

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

The initial model consisted of 2 one compartment neurons, each with 6 membrane currents. The equations describing a fast and a slow I_{Ca} and the hyperpolarization-activated inward current I_h were based upon voltage clamp recordings in HN cells. The other currents, I_{Na}, I_K (delayed rectifier) and I_A, were adapted from equations developed for molluscan neurons (Connor and Stevens, 1971, J. Physiol. 213: 31). Amplitude of synaptic conductance was determined by transmitter release, which was a function of presynaptic voltage.

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

416.17

SIMULATIONS OF PAVLOVIAN CONDITIONING IN NEURAL NETWORKS WITH ELEMENTS CONTAINING AN ADAPTIVE POTASSIUM A-CURRENT. J. Berner*, J. Gordon*, R. Pay, C.D. Woody (SPON: J. Barlow). UCLA Med. Ctr., Los Angeles, CA 90024.

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

416.18

SIMULATION OF 1000-NEURON INHIBITORY-FEEDBACK NETWORK REVEALS MEMORY PROPERTIES: COMPUTER MODELING INSPIRED BY NEOSTRIATUM. C.D. Myre, S.F. Sawyer, D.J. Woodward, *Dept. of Cell Biology and Anatomy, UT Southwestern, Dallas, Texas 75235.*

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

Support from the Biological Humanics Foundation, DA2338, and DA5352.

416.19

A COMPUTER SIMULATION ENVIRONMENT FOR IMPLEMENTING REALISTIC MODELS OF NEURAL SYSTEMS. M.A.Wilson*, U.S.Bhalla*, J.D.Uhley*, J.M.Bower, Division of Biology, Caltech, Pasadena CA 91125 (SPON: R.Lal)

We have developed a flexible neural systems simulator, written in C, running under the Unix operating system designed to facilitate the construction of neural models that are complex enough to provide data relevant to the organization and function of real neural networks using computers which are readily available to neurobiologists (c.f. Wilson and Bower, NIPS, AIP Press 1988). A graphics based front end using the X windowing system allows rapid, interactive specification and display of network circuitry and parameters. The built-in interpretive simulation language provides access to simulator structures and functions. The simulator is capable of generating output in the form of spike activity, EEGs, intracellular potentials and field potentials to facilitate the comparison of modeled results to physiological measurements. The hierarchical representation of components allows simulations ranging from single channels to detailed single cell models to large networks of simple or complex cells. The modular structure allows new components to be easily added to the existing library. Current models developed under this system include mammalian olfactory bulb and cortex, inferior olive, and invertebrate neural structures. (work supported by NSF grant EET-8700064, and the Lockheed corporation)

416.20

A LEARNING NEURAL-NETWORK MODEL OF THE OCULOMOTOR INTEGRATOR. D.B. Arnold* and D.A. Robinson (SPON: M.B. Sachs). Depts. of Ophthalmology and Biomedical Engineering, The Johns Hopkins Univ. Sch. of Med., Baltimore, MD 21205.

Single unit studies show that the extraocular motoneurons carry an eye position signal, whereas many oculomotor neurons in the brain stem encode only an eye velocity command. The eye position signal is derived from the eye velocity signal by means of a neural integrator located in the vestibular and prepositus hypoglossi nuclei.

Models of the neural integrator have been constructed which are robust and accurately reproduce its long time constant. The model neurons are described by first order linear differential equations and are connected by synapses hardwired in a reciprocal (feedback) inhibitory fashion that allows the network to process a signal varying in time.

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

417.1

THE EFFECT OF EARLY ENVIRONMENTAL ENRICHMENT ON DEWHISKERED RATS. K. Buhrmann*, R.C. Tees and L.A. Symons*. Department of Psychology, University of British Columbia, 2136 West Mall, Vancouver, B.C., Canada, V6T 1Y7.

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

417.2

EFFECTS OF NEONATAL SEROTONIN DEPLETION UPON DEVELOPMENT OF RAT SOMATOSENSORY CORTEX. J.A. Daugherty and J.H. Haring. Dept. of Anatomy & Neurobiology, St. Louis Univ. Sch. of Med., St. Louis, MO 63104.

Serotonergic (5HT) innervation of rat somatosensory cortex (SmI) is particularly dense in lamina IV during the early postnatal period (D'Amato et al., PNAS 84:4322, '87). The purpose of this study was to determine whether neonatal depletion of 5HT would influence development in SmI.

Rat pups received one subcutaneous injection of para-chloroamphetamine (PCA; 10mg/kg) on the day of birth and a second injection 24 h later. The brains of PCA-treated rats and control littermates were studied 20 d later using cytochrome oxidase histochemistry, Golgi impregnation or Nissl staining. Anterograde HRP labeling of thalamocortical axons was also accomplished in PCA-treated and control rats.

Three d after PCA treatment SmI 5HT is reduced to 35% of normal levels. Some recovery of SmI 5HT occurs but a permanent loss of 25% remains into adulthood. Despite the loss of 5HT, thalamocortical patterning in SmI appears normal in both cytochrome oxidase preparations and after anterograde HRP labeling. Likewise, barrel formation by lamina IV granule cells was normal. Preliminary analysis of apical dendrites of lamina V pyramids suggests that 5HT depletion results in fewer branches ($p<.001$) than in normal SmI. Since fewer dendritic branches could reflect delayed development intermediate time points are being studied.
Support: NS 25752 and DE 07734.

417.3

PATTTERNS OF RADIAL DENDRITES AND RADIAL GLIA IN BARREL CORTEX AFTER WHISKER REMOVAL IN NEONATAL MICE. J.E. Crandall, D.Butler*,V.S. Caviness, Jr. and J.-P. Misson. Departments of Developmental Neurobiology and Neurology, E.K. Shriver Center and Mass. Gen. Hosp., Waltham, MA 02254.

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

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

417.4

THE ROLE OF THALAMIC AFFERENCE IN THE DEVELOPMENT OF CEREBRAL CORTEX. M.S. Windrem, K.Rothe,* J.G. Schwartz,* and B. L. Finlay Dept. of Psychology, Cornell University, Ithaca, New York, 14853.

Functionally distinct regions of cerebral cortex vary in cytoarchitecture. Areas which receive massive thalamic afference tend to have more cells in a standardized cortical column. Much of this difference is due to a more prominent layer IV, which is the main recipient of thalamic input in sensory cortex. In normal posterior cortex, regions which have high cell death rates in the neonate have low cell number in the adult. Here we extend the investigation of the role of thalamic afference and neonatal cell degeneration in normal and experimentally deafferented cortex to the remaining cortical regions (prefrontal, motor, forelimb, hindlimb, somatosensory, and auditory).

On the day of birth, hamsters received unilateral electrolytic lesions of the thalamus. In experimental and litter-mate control animals, cells in columns from eight cytoarchitectonic regions of cortex were counted in adults and postnatal day 7 (P7) animals. Degenerating cells were counted throughout the entire neocortex in 12 equally-spaced sections of P7 animals.

For the eight regions, both cortical field and incidence of degenerating cells contributed to variation in adult cell number, with cell death inversely proportional to adult cell number. For all these areas, ablation of the innervating thalamic nuclei increased the incidence of degenerating cells in neonates and reduced cell number in adults.
Supported by NIH R01 NS19245 and F31 MH09576.

417.5

EMBRYONIC RAT NEOCORTEX TRANSPLANTED HOMOTOPICALLY INTO NEWBORN NEOCORTEX DEVELOPS AREA APPROPRIATE FEATURES. B.L. Schlaggar* and D.D.M. O'Leary (SPON: R. Grubb) Dept of Neurosurgery, Washington Univ Sch Med, St.Louis, MO. 63110

Embryonic rat visual cortex transplanted to the somatosensory region of newborn rats develops barrel-like morphologies. Barrels are aggregations of layer 4 stellate cells and thalamic afferents unique to rodent somatosensory cortex. We tested whether the observed barrel-like features are the result of transplantation per se by performing homotopic transplants of parietal and occipital cortex. A piece of cortex was removed from E17 donor fetuses which were exposed to 3H-thymidine in utero on E15. The donor cortex was placed in a cavity aspirated in a homotopic region in a newborn host. Hosts were perfused on P12 and alternate brain sections processed for AChE histochemistry which transiently reveals the afferent input from primary sensory thalamic nuclei, and Nissl staining which shows cortical cell distribution. In parietal cortex, AChE reveals the segregation of ventrobasal thalamic afferents, whereas in visual cortex it shows the homogeneous geniculocortical termination pattern. Aggregations of layer 4 stellate cells correspond with the disjunctive pattern of intense AChE staining in somatosensory cortex. In visual cortex, the continuous band of layer 4 stellate cells corresponds to the homogeneous pattern of AChE. Transplants are delineated by autoradiography of adjacent sections. Homotopic parietal and heterotopic occipital-to-parietal transplants form barrel-like structures (i.e. aggregations of layer 4 neurons coincident with intense AChE staining) equally well. However, homotopic occipital transplants do not contain barrel-like morphologies. These results suggest that transplantation per se does not cause the development of barrel-like morphologies. Rather, embryonic occipital or parietal cortex transplanted to the neonatal parietal region form barrel-like structures due to the influence of ventrobasal thalamocortical afferents. (Supported by NINCDS grant 5 P01 NS17763 & The McKnight Foundation)

417.6

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

Type II calcium/calmodulin-dependent protein kinase (CaM kinase) is one of the most abundant brain protein kinases, and is particularly concentrated in neocortex and hippocampus, where it may regulate a variety of specialized functions. We used immunocytochemical techniques to examine the distribution of kinase-containing neurons in rat somatosensory cortex, a highly organized, well-characterized region suitable for future studies of activity-dependent regulation. Kinase-immunoreactive neurons are present throughout laminae II-VI; both pyramidal and non-pyramidal neurons are stained. Within some layers, the distribution of kinase-positive neurons is not homogeneous. A subtle pattern of alternating light- and dark-staining bands is present in sections taken through layer IV, in the vibrissal "barrel" subfield. Bands of lighter CaM kinase immunostaining correspond to cytochrome oxidase-intense rows of barrels, while darker immunostaining is present in the areas between rows. The difference in kinase staining intensity between the two regions is due to a difference in density of immunoreactive layer IV neurons, and of the apical dendrites of kinase-positive infragranular pyramidal cells cut in cross-section. This suggests that CaM kinase-immunoreactive pyramidal cells in layers V and/or VI are distributed in a pattern related to that of layer IV neurons.

417.7

THE DISTRIBUTION OF THALAMIC PROJECTION NEURONS IN DEVELOPING RATS. K.E. Forni* and L.-Y.M. Huang (SPON: F.H. Rudenberg). Marine Biomedical Institute, University of Texas Medical Branch, Galveston, TX 77550.

The distribution of thalamic projection neurons has been studied in rats ranging in age from postnatal day 2 (P2) to postnatal day 15 (P15). The projection neurons were identified by the retrograde transport of fluorescent latex microspheres following injections of microspheres on one side of ventral posterolateral (VPL) and/or ventral posteromedial (VPM) thalamus nuclei. The brain and spinal cord were removed from the animals after at least 48 h survival time. The tissue was frozen and sectioned transversely at 48-64 µm on a cryostat. We have shown previously that the pattern of distribution of the projecting neurons in P14-P18 rats is very similar to the pattern observed in adult rats (Brain Res., in press). The distribution of thalamic projection neurons in P1-P12 rats is different from that seen in adult rats. The projection neurons are not confined to a few defined nuclei, but are dispersedly distributed. The total number of projection neurons increases substantially during the first 10 days of postnatal life. These results suggest that thalamic projections are not complete at birth. During the first 2 weeks of postnatal development, a great deal of rearrangement of thalamic projections occurs. Some of the projections are eliminated while other projections arrive at their target areas. Supported by NS23061 and NS01050.

417.8

DEVELOPMENT OF SI FORELIMB BARREL REPRESENTATION IN NORMAL AND DEAFFERENTED NEONATAL RATS AS STUDIED USING PEANUT AGGLUTININ (PNA). C.A. McCandlish and R.S. Waters. Dept. of Anatomy and Neurobiolology, Univ. of Tennessee, Memphis, Col. of Medicine, Memphis, TN 38163.

The lectin, Arachis hypogaea, peanut agglutinin (PNA) has been used to study early pattern formation of the face barrel subfield representation in the somatosensory cortex. Using PNA, we extended those findings to include the entire body representation, and provided evidence for differential development between the face and limb subfield representations. In this report, we describe the role that afferent input plays on early pattern development of the forelimb barrel subfield representation.

Rat pups ranging from PND-1 through PND-8 were anesthetized, and the left forearm was removed. The skin was sutured, and the pup returned to the dam. On PND-8, pups were anesthetized with sodium pentobarbital and perfused with buffered saline followed by 4% paraformaldehyde and 2% glutaraldehyde in 0.2 M sodium cacodylate buffer. Brains were removed, hemispheres were separated, and flattened tangentially. The tissue was vibratome sectioned, placed in 2% bovine serum albumin, incubated in PNA at 4°C overnight and reacted by means of peroxidase histochemistry. Lectin-bound barrel subfields were then compared between the two hemispheres.

Deafferentations made between PND-1 and PND-4 resulted in an aberrant pattern of forelimb representation on the hemisphere contralateral to the amputated limb. The resulting pattern consisted of conspicuously absent digit and limb representation boundaries, or abnormal boundary patterns. These patterns showed variations within and between animals deafferented on the same postnatal day. The ipsilateral limb showed a normal pattern in all cases examined. Pups deafferented after PND-4 showed no differences in the forelimb representation between hemispheres.

These findings provide evidence for a critical period of development when afferent input is necessary to shape the underlying substrate into the adult pattern of barrel-field organization. (Supported by NSF Grant BNS 88-02766.)

417.9

DECREASED SEROTONIN CONTENT IN RAT SOMATOSENSORY CORTEX AFTER NEONATAL INFRAORBITAL NERVE LESION. J.H. Haring[1], C. Bennett-Clarke[2], R.W. Rhoades[2] & M.F. Jacquin[1]. St. Louis Univ. Sch. of Med.[1], St. Louis, MO 63104 and Med. Coll. of Ohio,[2] Toledo, OH 43699.

Serotonergic projections to rat SmI form discontinuous patches during the first 3 postnatal wks (D'Amato et al., PNAS 84:4322, '87). These patches appear to correspond to the pattern of thalamocortical afferents (TCA) to SmI. The organization of TCA in the vibrissa pad region of SmI (PMBSF) is disrupted by infraorbital nerve section on the day of birth. The purpose of this study was to determine if TCA reorganization would influence the PMBSF 5HT plexus.

Transections of the left infraorbital nerve were performed on rat pups on the day of birth and PMBSF tissue was harvested for HPLC analysis of 5HT and 5HIAA on postnatal days (PND) 7,12, and 17. On PND7, 5HT levels were about equal in the right (altered TCA) and left (normal) PMBSF. Right PMBSF 5HT was decreased slightly compared with the left in PND 12 rats. By PND17, right PMBSF 5HT content was reduced to about 50% of left 5HT levels. Changes in 5HIAA levels were equivalent to the changes observed in 5HT content at each postnatal period. Turnover rates indicated significant increases in 5HT neuronal activity on PND12 and 17, but turnover was not different between right and left PMBSF. These data suggest that the changes in right PMBSF 5HT levels are not due to changes in raphe neuronal activity but are perhaps the result of an altered 5HT axon plexus. Support: NS25752 and DE 07734.

417.10

SINGLE-DIGIT-DENERVATION-INDUCED IMMEDIATE PLASTICITY IN SOMATOSENSORY CORTEX IS BILATERAL. M.B. Calford and R. Tweedale. Vision, Touch and Hearing Research Centre, Department of Physiology and Pharmacology, University of Queensland, St. Lucia, Qld. 4067, Australia.

In adult mammals a small amputation, such as that of the thumb in a flying-fox or a toe in a rat, usually produces a rapid expansion of receptive fields at primary somatosensory (SI) cortical loci which originally represented the denervated area. This expansion is also seen when a small body region is locally anesthetized. Here we report that in anesthetized flying-fox not only is the representation of the denervated region in contralateral SI affected but that the representation of the mirror image body region, in cortex ipsilateral to the denervation, also shows an expansion. In normal physiological conditions we cannot show an effect of stimulation upon ispilateral SI in the form of direct excitation, direct inhibition or competitive inhibition. The expansion and contraction of the receptive fields within the representation of the body region contralateral to that denervated shows the same time-course as the direct effect in those cases where local anesthetic was used to create a temporary effect; when amputation was used the ipsilateral expansion also retracted although the period of expansion varied considerably. One simple aspect of the result which has consequences for the general understanding of plasticity in adult brain is that under conditions where conventional physiology could not demonstrate the influence of a pathway that pathway has been shown to have an inhibitory influence upon viable inputs and indeed is one of the influences which shape receptive fields.

SOMATOSENSORY CORTEX AND THALAMOCORTICAL RELATIONSHIPS III

418.1

ORGANIZATION OF NOCICEPTIVE SI NEURONS IN THE PRIMATE. W.C. Perkins* and D.R. Kenshalo, Jr. NAB, NIDR, NIH, Bethesda MD, 20892 and Division of Neurobiology, Barrow Neurological Institute, Phoenix, AZ 85013

Previous studies have reported a population of nociceptive neurons in the primary somatosensory cortex (SI) of anesthetized monkeys. However, the laminar distribution and somatotopic organization of nociceptive neurons remains unclear. Single neurons were recorded in SI cortex of young, adult monkeys anesthetized with alpha-chloralose and supplements of sodium pentobarbital. The receptive fields of nociceptive cortical neurons were classified as either wide-dynamic-range (WDR) or nociceptive specific (NS). The average receptive field areas of WDR neurons (mean=1897 mm^2) were significantly larger than NS neurons (mean=770 mm^2). Nociceptive neurons were somatotopically organized in SI. Those neurons with receptive fields on the foot were located more medial in SI than those with the receptive fields on the hand. NS neurons were only found in the anterior half of the distribution of nociceptive cells, while WDR neurons were distributed over a wider area of SI. The majority (81%) of nociceptive neurons were located in the middle layers of SI. Once a nociceptive neuron was encountered, small movements of the microelectrode resulted in a shift in the location of the receptive field, but nociceptive neurons were still isolated. These data suggest that nociceptive SI neurons are found in aggregations or clusters in the middle layers of SI and are somatotopically organized.

418.2

MODULATORY EFFECTS OF NOREPINEPHRINE ON RESPONSIVENESS OF CAT SOMATOSENSORY CORTICAL NEURONS. R.W. Dykes[1] and R. Warren[2]. [1]Dept. of Physiol., Univ. of Montréal, Montréal, Canada, H3C 3J7 and [2]Dept. Neurol. Neurosurg., McGill Univ., Montréal, Canada, H3B 4I4.

Norepinephrine (NE) was administered to neurons in cat somatosensory cortex in an effort to determine the effects of NE on neuronal excitability. Under halothane anesthesia, the somatosensory cortex was exposed and a multibarrel microiontophoretic pipette was introduced. From 59 cats, 368 neurons were held long enough to collect information about receptive field location, depth in the cortex and sensitivity to microiontophoretically administered glutamate. In this sample, 26% displayed peripheral receptive fields, 25% showed some afferent input during glutamate administration whereas for 49% we were unable to demonstrate the presence of peripheral input. Cells with receptive fields displayed spontaneous activity more frequently than cells without receptive field (57% vs 24%).

In general the effect of NE was to suppress both spontaneous and evoked activity. In a sample of 63 neurons, 26/42 that had spontaneous activity had their ongoing activity reduced in a greater proportion than the evoked activity, resulting in an increase in signal-to-noise (S/N) ratio. In 10 cases NE produced a decrease and in 4 cases there was no change in S/N ratio. In the majority of cells where spontaneous activity was absent, NE caused a reduction of activity whether it was evoked by somatic stimulation or by glutamate microiontophoresis and few cells appeared to be insensitive to NE.

The major action of NE in the somatosensory cortex seems to be an attenuation of neuronal excitability, this action being stronger on weak inputs than on strong inputs evoked either by peripheral stimulation or glutamate pulses, resulting in an increase in S/N ratio. (Supported by MRC of Canada).

418.3

PROLONGED EFFECTS OF NOREPINEPHRINE ON RESPONSIVENESS OF CAT SOMATOSENSORY CORTICAL NEURONS. R. Warren[2], L. Buyan*[2] and R.W. Dykes[1]. [1]Dept. of Physiol., Univ. of Montreal, Montreal, Canada, H3C 3J7 and [2]Dept. Neurol. Neurosurg., McGill Univ., Montréal, Canada, H3B 4L4.

The study of norepinephrine (NE) described in the abstract by Dykes and Warren (1989) was extended to examine the possibility that there may be long-term effects on neuronal excitability resulting from the treatment of neurons in somatosensory cortex with NE.

In the sample of 368 neurons isolated from the forelimb cortex of halothane anesthetized cats, 54 cells were studied for variable periods of time after the end of NE treatment. Neuronal excitability was tested regularly with somatic stimuli or with a pulse of glutamate, the timing being controlled by a computer. The impulses were converted to digital signals and stored on a computer. These responses were taken as measures of neuronal excitability and were compared to the responses during the control period that preceeded NE treatment.

Thirty one (58%) of these neurons showed an enhancement of excitability following the end of NE treatment of which 6 (over 13) had receptive fields and 25 (over 41) with no receptive fields. The effect developed within seconds to a few minutes of the end of NE administration and was generally more pronounced in the presence of glutamate. When cells were studied for longer periods of time, the enhanced responsiveness appeared to remain or to decline only very slowly and often lasted for as long as the cell was held. The role that such a change in excitability might play in cortical function has yet to be clarified. (Supported by MRC of Canada).

418.4

CHANGES IN RECEPTIVE FIELD ORGANIZATION OF PRIMATE SI NEURONS DURING IONTOPHORESIS OF A GABA ANTAGONIST. K. Alloway and H. Burton. Department of Anatomy & Neurobiology, Washington University School of Medicine, St. Louis, MO 63110

We characterized the receptive field (RF) properties of SI neurons to punctate stimuli before and during iontophoresis of bicuculline methiodide (BMI) in macaque monkeys that were anesthetized with either pentobarbital or nitrous oxide and halothane. Neurons in areas 3b and 1 having glabrous skin representation throughout the digits and palm were activated by constant amplitude indentations applied sequentially to multiple points in the RF.

Some slowly-adapting (SA) neurons responded to BMI with an expansion of RF size. Many SA neurons, however, failed to exhibit RF expansion and were also resistant to the inhibitory effects of GABA.

Rapidly-adapting (RA) responses were inhibited by iontophoretic application of GABA and this inhibitory effect was subsequently antagonized by BMI. During BMI iontophoresis, RA neurons usually exhibited an expansion of the RF into neighboring regions including digits that were outside the original RF. Contour maps frequently indicated that the pattern of RF expansion across digits was most pronounced at noncontiguous sites that would normally be juxtaposed to the most sensitive site in the original RF. We conclude that the geometry of the expansion tended to incorporate those parts of the skin that normally would be activated together during manipulation of the hand. Supported by NIH NS09809 and 22012.

418.5

RESPONSIVENESS OF REORGANIZED SOMATOSENSORY CORTEX (SI) AFTER INACTIVATION OF NORMAL SI CORTEX IN CHRONIC SPINAL CATS. C. Casanova, P. A. McKinley, C. Chau*, S.Molotchnikoff. McGill University, School of Physical and Occupational Therapy, Montréal, Canada, H3G 1Y5.

Major reorganization of the cortical somatotopic map occurs in cats deprived of hindlimb afferent input at 2-weeks of age (McKinley et al., Develop. Brain Res. 31:136, 1987). In these animals, the region normally devoted to the hindlimb becomes devoted to the trunk and forelimb and the somatotopic maps are characterized by two representations of the trunk and forelimb. We have investigated the possibility that intracortical connections are the anatomical substratum providing new input to the deprived cortex. Experiments were carried out on anesthetized adult cats that were cord-transected (T_{12}) at 2-weeks of age. Carbon fiber-filled micropipettes were used to record multi-unit activity. The cortex was mapped and the normal and new representations of the forelimb and trunk were clearly identified. The recording electrode was kept in the reorganized region while an injecting-recording micropipette filled with stained lidocaine was placed in a somatotopically corresponding region of the normal cortex. Our results indicate that the inactivation of the normal cortex failed to reduce the responses of the reorganized cortex. These results suggest that intracortical connections from the "normal" somatotopic sites are not the means by which the "novel" somatotopically corresponding sites are driven.

418.6

PLASTICITY OF RACCOON SOMATOSENSORY CORTEX: ROLES OF CONVERGENT SENSORY INPUTS AND CORTICO-CORTICAL EPSPS E. Smits*, D.C. Gordon*, P. Zarzecki and D.D. Rasmusson. Dept. of Physiology, Queen's Univ., Kingston, Ontario, K7L 3N6 and Dept of Physiology & Biophysics, Dalhousie Univ. Halifax, N.S., B3H 4H7, Canada

We tested two hypotheses: 1) that multidigit inputs are present in primary somatosensory cortex of raccoons and 2) that corticocortical connections are enhanced during plastic remodeling of cortex. Intracellular recordings were made, under barbiturate anesthesia, from 104 neurons in the representation for glabrous skin of digit four (D4) in two age-matched groups. In controls, electrical stimulation of D4 glabrous skin evoked epsps in 98% of neurons. However, inputs did not originate only from D4, since D3 or D5 stimulation produced epsps in 28% of these neurons. Six to nine months after removal of D4, the proportion of neurons with inputs from D3 or D5 was increased to 76%. Microstimulation (ICMS) in the rostral "heterogeneous" cortex evoked corticocortical epsps in 73% of neurons in controls and in 90% of neurons after amputation.

Epsps can be evoked from multiple digits and by a corticocortical path in normal cortex. The corticocortical path could relay the inputs from adjacent digits. Stronger corticocortical epsps after deafferentation could account for the new sensory properties of somatosensory cortex.
Supported by the Medical Research Council of Canada.

418.7

DIFFERENTIAL RESPONSIVENESS OF SII FOLLOWING SI LESIONS IN INFANT AND JUVENILE MACAQUES. K. Sathian, Shao Dian-Hua* and H. Burton. Dept. of Anatomy and Neurobiology, Washington University School of Medicine, St. Louis, MO 63110.

Pons et al (Science 237: 417-420, 1987) reported that lesions in primary somatosensory cortex (SI) of adult macaques abolish responsiveness in the corresponding representation in second somatosensory cortex (SII). We ablated the hand representation in SI in 2 juvenile and 3 infant (4-6 weeks postnatal) Macaca mulatta and recorded in ipsilateral SII (one hemisphere per animal). Substantial portions of the SII hand area were unresponsive. However, in each animal, some responses could be evoked in SII by mechanical stimulation of the hand. Relatively few responses were elicited by low-threshold cutaneous stimuli and no cutaneous receptive fields were restricted to the digits. Representations of body parts other than the hand were normally responsive and their location was consistent with normal somatotopy in SII. The proportion of cutaneous receptive fields was higher in neonatally-lesioned macaques than in the older animals. This may explain the greater tactile capacity of the former (Carlson and Burton, J. Neurosci. 8: 833-859, 1988). In the juveniles the SI lesions spared small parts of areas 3a and 3b, which could have contributed to the residual somatic drive. However, in the neonatally-lesioned monkeys this was not the case. Further, in one infant, injection of Fast Blue into SII at a site where cutaneous input was recorded from the hand retrogradely labeled cells in motor cortex and VPL, VPI and PO thalamic nuclei, but not in SI. Supported by NIH NS 09809 & NS 15070.

418.8

PARALLEL PROCESSING IN THE FIRST AND SECOND SOMATOSENSORY AREAS IN TREE SHREWS. P.E. Garraghty, S.L. Florence, W.N. Tenhula, and J.H. Kaas. Department of Psychology, Vanderbilt University, Nashville, TN 37240.

In monkeys, the second somatosensory area (SII) depends on inputs from anterior parietal cortical areas 3a, 3b, 1, and 2 ("SI") for its activation. Thus, if the hand representation of SI is removed, stimulation of the hand no longer drives neurons in SII. In contrast, neurons in SII of cats are unaffected by the removal of SI cortex. Because of this dramatic species difference, we are continuing a comparative analysis, and report here on the effects of SI lesions on the responsiveness of neurons in SII in the tree shrew. In these experiments, we first mapped, in anesthetized subjects, the forelimb representation in SII. We next mapped the forelimb representation in SI and then lesioned it by aspiration. We then remapped the SII hand representation. In all 5 cases, neurons in SII with receptive fields on the forelimb remained highly responsive to cutaneous stimuli after the lesion. These results, together with those mentioned earlier for other species, suggest that SI and SII are independently activated by thalamic projections in most mammals and that a dependency of SII on SI may be present only in monkeys, emerging relatively recently in evolution. We suggest that the pattern of thalamocortical relations with SI and SII can be used to predict whether or not a dependency exists. (NS16446)

418.9

SINGLE NEURON RESPONSE PROPERTIES IN SENSORIMOTOR CORTEX IN AWAKE CATS. J.C. Slimp and A.L. Towe. Dept. of Physiology and Biophysics, Univ. of Wash., Sch. of Med., Seattle, WA 98195

Three quasi-planar arrays of 10 closely-spaced microelectrode tracks were run in primary somatic and pericruciate sensorimotor forelimb cortex in two awake, passively restrained domestic cats. Guide chambers for the electrode carriers were affixed to the cranium while the cats were under deep barbiturate anesthesia. The modality sensitivity, receptive field (RF) location and size, adaptation rate, and state of arousal were recorded for 504 neurons isolated at 337 sites (2 or more neurons were isolated at 120 sites). The spatial distribution of these response properties failed to show any clear organization, other than somatotopy. Quantitative tests of the predictions of a strict columnar and of a random distribution model showed no difference from the random model, in all three arrays. Few simultaneously-recorded neurons shared the same modality and RF; 2/3 had limited or no RF overlap and 5/9 showed different modality sensitivities. Many pairs with modality sharing showed limited or no RF overlap, and many pairs with partial or complete RF overlap did not respond to the same modality. The data failed to support any model featuring local bounded regions within which modality sensitivity and RF location and size are the same. Local clusters sharing the same response properties were not excluded.

418.10

AN ANATOMO-PHYSIOLOGICAL STUDY ON THE FUNCTIONAL MATCHING BETWEEN RECEPTIVE FIELDS OF SII NEURONES AND ASSOCIATION INPUT FROM SI IN CATS. P.Barbaresi, S.Bernardi*, T.Manzoni.* Inst. of Human Physiology,University of Ancona,Italy.

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

418.11

BILATERAL INTERACTION IN CAT SII AND THE CORPUS CALLOSUM. N. Picard*, F. Lepore, M. Ptito, J.-P. Guillemot.Dépt.de Kinanthropologie et de Psychologie, Université du Québec et de Montréal, H3C 3P8.

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

418.12

CONVERGENT INPUTS TO SINGLE NEURONS IN SmI FOREPAW DIGIT CORTEX OF RACCOONS. G.S.Doetsch, S.D.Stoney, Jr. and D.H.Hauge*. Dept. of Surg. (Sect. of Neurosurg.) and Dept. of Physiol. & Endocrinol., Med. Coll. of GA, Augusta, GA 30912

Neurons in digit 3 and digit 4 cortex of chloralose-anesthetized raccoons were tested for afferent convergence using mechanical and electrical stimulation of all digits in conjunction with condition-test (CT) interactions. Most neurons received strong facilitatory or weak excitatory influences from several off-focus digits as well as excitatory input from the on-focus digit. Strongest facilitation typically occurred with CT intervals of 1-5 msec. Off-focus excitatory responses usually had higher thresholds, lower probabilities, fewer spikes and longer latencies (1-10 msec) than on-focus responses. Neurons located in "heterogeneous" hairy skin representations had larger receptive fields (RFs) and were facilitated from more digits than neurons in glabrous skin representations. Application of the GABA antagonist picrotoxin sometimes enlarged excitatory RFs to include several off-focus digits. Unmasking or strengthening of convergent inputs may account for the SmI reactivation observed following peripheral nerve lesions (Kelahan & Doetsch, 1984). Supported by NSF Grant BNS-8419035.

418.13

THE EXCITATORY AMINO ACID ANTAGONIST (EAAA), MK-801, ATTENUATES SOMATOSENSORY-EVOKED POTENTIALS (SEPs) OF RATS. T.L. SAILER*,T. EMREY* AND R.R. NOTVEST. Wyeth-Ayerst Research, Princeton, NJ 08543-8000

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

418.14

VIBRISSECTOMY CAUSES TRANSIENT CHANGES IN GAP-43 IMMUNOREACTIVITY IN THE ADULT RAT BARREL CORTEX. K. Wood*, [1]L. Benowitz, A. Dunn-Meynell and B. Levin (SPON: S.D. Cook), Neurology Svc., VA Med. Ctr., E.Orange, NJ 07050, [1]Dept. Psych., Harvard Med. Sch., McLean Hosp., Belmont,MA 02178.

Removal of mystacial vibrissae in adult rats leads to alterations in the representation of the vibrissae in the barrel cortex. We examined changes in the distribution of growth associated protein, GAP-43 immunoreactivity (GAP-IR) induced by vibrissectomy (vbx) as a possible index of sprouting. All but the central (C3) vibrissae were removed unilaterally from adult male SD rats. 6D or 14D later, the barrel cortices were removed, flattened, sectioned tangentially and reacted to reveal GAP-IR by immunocytochemistry. In layer IV of the cortex, barrel centers contained low GAP-IR, whilst the inter-barrel regions had higher GAP-IR. The area of the C3 barrel center defined by GAP-IR was unchanged by spared C3 vbx at either survival time. However at 6D post surgery, the average area of the deafferented barrels surrounding C3 decreased by 10.0% relative to the control side ($P<0.025$) whilst the interbarrel area showed a non-significant increase (4.6%, $P>0.10$). No such changes were apparent with 14D survival. These data provide evidence for structural remodeling in the adult brain, suggesting that GAP-43 containing terminals sprout into barrel centers after vbx induced deafferentation of the barrel cortex. Supp. by the VA Medical Research Svc. and NS25830.

418.15

TRANSIENT EXPRESSION OF GAP-43 IN THE DEVELOPING RAT BARREL FIELD CORTEX. R.S. Erzurumlu, S. Jhaveri* and L.I. Benowitz, M.I.T., Cambridge, MA, 02139 and Harvard Medical School, McLean Hospital, Belmont MA 02178.

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

GAP-43 was first evident in the differentiating layer IV on PND3 as an array of punctate densities which correspond to the pattern of vibrissae on the snout, and which are confined to prospective barrel hollows. The densities were most intense on PND5, at which time they were surrounded by newly emerging cell-dense barrel walls. Towards the end of the first postnatal week, the staining intensity in the barrels dramatically decreased, while the size of immunopositive densities increased and was comparable to that seen with CO. By PND8, GAP-43 immunostaining had virtually disappeared from the barrels, but was dense in the septa. This staining pattern was complementary to that seen with CO, and persisted into adulthood. Following partial or total row C vibrissae cautery on PND0, the distribution of GAP-43 densities representing the damaged whiskers formed a continuous band. This pattern was seen as early as PND3.Based on the pattern and time course of GAP-43 expression and on the effect of peripheral manipulations, we conclude that the GAP-43 staining reflects the distribution of ingrowing thalamic afferents.Thus, the disjunctive organization of these afferents occurs prior to cytoarchitectonic differentiation of cortical barrels.

Supported by NIH grants EY05504, EY00126 and NS25830.

418.16

SURVIVAL OF LABELED THALAMOCORTICAL NEURONS IN TISSUE CULTURE. L.M. Smith and K.A. Jones, Center for Neural Science, Brown University, Providence, R.I. 02912 and Department of Neurobiology, Harvard Medical School, Boston, MA 02115.

Unlike many types of CNS neurons, thalamic projection neurons have been difficult to grow *in vitro* for extended periods. We attempted to optimize thalamic neuronal survival in culture by studying the 1) method of labeling the cortically projecting neurons, 2) age and method of dissociation, 3) source of glial feeder layer and 4) method of reducing excitotoxic effects. Thalamocortical neurons were prelabeled *in vivo* by retrograde transport of fluorescent latex microspheres. The parietal cortex of rat pups was injected with .2µl label through the uterine wall of the mother two days before birth. Within three hours after birth, the dorsal thalamus was dissected from the newborn brain, dissociated enzymatically using the enzyme papain and plated as a suspension of single cells (130 cells/mm^2) on a feeder layer of thalamic and/or cortical astrocytes (Huttner and Baughman '88). The excitatory amino acid antagonist, kynurenate (1 mM), was added to the dissociating and growth mediums to protect thalamic neurons from glutamate excitotoxicity (Choi et al., 1988).

Twenty four hours after plating most cells had a distinct bipolar or multipolar neuronal morphology; 4.0% of these cells were densely labeled. Three weeks after plating, 48% of the initially plated neurons survived, and 4.3% of these were still labeled with microspheres, suggesting that thalamocortical projecting neurons survived roughly in proportion to the number originally plated. Thus these conditions are sufficient to support prolonged survival of thalamocortical neurons in culture, and will allow future studies of their synaptic physiology. (Supported by NS13031 and EY03502).

418.17

NAAG-POSITIVE NEURONS AND TERMINALS IN THE SOMATIC SENSORY CORTEX OF RATS AND CATS. F. Conti, J.H. Neale§, T. Manzoni* Inst. of Human Physiol., Univ. of Ancona, Ancona (Italy); §Dept.of Biol., Georgetown Univ., Washington, D.C (USA).

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

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

VISUAL CORTEX V

419.1

EFFECT OF SEQUENTIAL IMAGE PRESENTATION ON TEMPORALLY MODULATED RESPONSES OF STRIATE CORTICAL NEURONS. J. M. Watanabe*, T. J. Gawne*, B. J. Richmond, and L. M. Optican*. Laboratory of Neuropsychology, National Institute of Mental Health, and Laboratory of Sensorimotor Research, National Eye Institute, Bethesda, Maryland 20892.

The responses of neurons in primary visual cortex show stimulus-dependent temporal modulation: when a stimulus is presented in isolation, the waveform of the response depends upon the spatial pattern and duration of the stimulus. However, images do not occur in isolation during normal vision. Thus, it is important to know how changes from one image to another affect the responses of visual neurons. In this study, responses to stimuli consisting of sequential images were compared to the responses to isolated images.

Single units in primary visual cortex were recorded in behaving monkeys. During fixation, 2-dimensional black and white stimuli were flashed on the neuron's receptive field. Stimulus pairs that gave disparate response waveforms were chosen. Each stimulus was presented in isolation for each of 18 durations. The stimuli were also shown sequentially, with varying durations (33 - 133 msec) and varying intervals between them (0 - 133 msec). The responses to the paired stimulus presentations were compared with the predictions of a superposition model, based on the linear addition of the responses to each stimulus presented alone.

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

419.2

ESTIMATING INFORMATION TRANSMITTED BY NEURONS. T. J. Gawne*, L. M. Optican*, and B. J. Richmond. (SPON: J. Carl) Lab. Neuropsychology, NIMH and Lab. Sensorimotor Research, National Eye Institute, Bethesda, MD 20892.

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

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

$\mathbf{T}^*$ is accurate within 5% for sample sizes as small as 7 in simulated data sets contaminated with both Gaussian and uniform noise. For neuronal data, $\mathbf{T}^*$ was a better estimator than **T** for sample sizes below 30. Thus, we propose $\mathbf{T}^*$ as a new estimator for transmitted information in biological signals since it minimizes errors caused by 1) quantization, 2) noise, and 3) small sample sizes.

419.3

LINKING SIMPLE CELLS WITH QUADRATIC MODELS OF MOTION PERCEPTION. H. Suarez* & C. Koch (SPON: M. Kennedy). Computation and Neural Systems, Caltech, Pasadena, CA 91125.

Behavioral experiments on insects as well as psychophysical evidence (Hassenstein & Reichardt, 1956; Van Santen & Sperling, 1985) support the notion that short-range motion perception is mediated by a system with a quadratic type of nonlinearity. However, there is little physiological evidence for quadratic nonlinearities in directionally selective cells. Both simple and complex cells of visual cortex appear to be linear in stimulus contrast, for low contrast (Holub & Morton-Gibson, 1981). Moreover, known biophysical mechanism do not seem to have quadratic properties, except under limiting conditions (Grzywacz & Koch, 1987). We discuss here a simple mechanism offering a solution to this problem, as well as a detailed model of simple cells. We assume a population of directionally selective cells with random thresholds (distributed uniformly in an interval) and a linear response above these thresholds, until saturation. In a linear threshold function, increasing the threshold is equivalent, with respect to the output, to decreasing the input of the same amount. The sum of the population responses to a given input, indicative of the perceptual response, is then the same as the sum of the responses of a single neuron with the lowest threshold to a group of inputs distributed uniformly in an interval. The perceptual response is thus approximately the integral of the linear neuronal response curve, as in Monte-Carlo integration, and proportional to the square of the input. Simulations with 50 neurons fit a square function well.

419.4

A BACKPROPAGATION TRAINED MODEL SIMULATES THE RESPONSES OF DISPARITY TUNED PRIMARY VISUAL CORTEX NEURONS. D. Zipser, Cognitive Science Dept., Univ. of Calif. San Diego, La Jolla, CA 92093.

Backpropagation learning has proven useful in programming model neural networks to simulate the observed responses of actual cortical neurons. While backpropagation is a supervised learning procedure, it can be used in an unsupervised mode, called identity mapping, in which the output target is exactly the same as the input pattern. The responses of the hidden units in backpropagation trained identity mapping networks represent an optimal feature encoding of the input patterns closely related to the principal components of the input correlation matrix. A model neural network was trained by backpropagation to do an identity mapping using as input a pair of retinas on which a stimulus was represented with a range of disparities. The disparity tuning of the hidden units in this network was compared to those found experimentally in areas 17 and 18 of the cat (LeVay, S. and Voigt, T. *Visual Neurosci.*, 1:395, 1988). The cat has two classes of disparity tuned neurons: one with narrow excitatory response functions tuned to very small disparity, the other broadly tuned to respond mostly to stimuli either in front of or beyond the fixation point. All the hidden unit disparity tuning patterns found in the backpropagation trained network are also found in the brain, and all of them correspond to the broadly tuned cortical neurons. No hidden units that matched the sharply tuned neurons were found.

419.5

RECEPTIVE FIELDS OF GENICULOCORTICAL AFFERENTS TEND TO BE ALIGNED ALONG PREFERRED ORIENTATION OF CORTICAL CELLS. B. Chapman*, K.R. Zahs*, & M.P. Stryker (SPON: A.J. Hudspeth). Dept. Physiol. & Neurosci. Grad. Program, Univ. California, San Francisco, CA 94143-0444.

In earlier experiments (*J. Neurophysiol. 59*:1410, 1988), we noted that the geniculocortical afferent receptive fields encountered in radial electrode penetrations through area 17 were commonly dispersed so as to cover elongated regions of the visual field. We now attempt to relate the axis of this elongaton to the orientation of the cortical cells recorded along the same penetration. Radial penetrations were made into visual cortex in normal adult ferrets and the orientation preference of the cortical cells was recorded. Cortical cell responses were then eliminated by superfusion of the cortex with either kainic acid, an excitatory neurotoxin, or muscimol, an agonist of the inhibitory neurotransmitter GABA. After drug treatment, we were able to record from many single units with distinct receptive fields. These units are presumed to be geniculocortical afferent terminals because their responses were characteristic of geniculate cells and their recording sites were found to be within layer IV in all penetrations reconstructed to date. The set of single-afferent receptive fields covered an elongated region of the visual field in each of 7 penetrations (2 kainate, 5 muscimol); in all of these cases, the line formed by the afferents' receptive fields paralleled the preferred orientation of the cells recorded within the penetration. In 3 penetrations (2 kainate, 1 muscimol), the afferent receptive fields did not cover an elongated region of the visual field, although the cortical cells in these penetrations were as orientation selective as those in the other 7 penetrations.

These results may throw light on the arrangement by which simple cells become selective for stimulus orientation.

Supported by grants from the N.I.H., the McKnight Foundation, and a UCSF Graduate Opportunity Fellowship.

419.6

IPSI-THALAMUS SUPPORTS MOST ACTIVITY IN THE IPSI-HEMIFIELD REPRESENTATION AT THE 17-18 BORDER. E.L. Noll* and J.G. Malpeli, Dept. Psych., Univ. Illinois, Champaign, IL 61820.

Receptive-fields at the cat 17-18 border extend at least 5° further into the ipsi-hemifield than those in the LGN. If all cortical activity were driven by the LGN's, all ipsi-hemifield activity would be due to callosal inputs. While callosal inputs have been shown to drive cells at the 17-18 border, we have previously found that not all cells in visual cortex depend on the LGN. In this study we examined the contributions of direct and callosal inputs to ipsi-hemifield activity. After locating such cells in cortex, we found the maximum extent of ipsi-fields in the LGN at the same elevation, and placed injection pipettes under the LGN near this site in both optic tracts. Since simultaneous injections of lidocaine under both LGN's reversibly silenced cortical activity, we were able to unequivocally determine the fraction of cortical activity supported by direct thalamic or callosal input. On average, direct thalamic inputs were capable of supporting 88% of normal activity, whereas callosal inputs supported 22%. Control experiments suggest that the medial interlaminar nucleus (MIN) is the source of most ipsi-hemifield activity along the 17-18 border. The fact that the MIN relays ipsi-hemifield activity only for the contra-eye may explain why only the contra-eye receptive fields extended well into the ipsi-hemifield, even though most cells in this study were binocular. (Supported by NIH grant EY02695)

419.7

RESPONSES OF NEURONS IN PRIMARY VISUAL CORTEX ARE INFLUENCED BY EYE POSITION. T.G. Weyand and J.G. Malpeli. Department of Psychology, University of Illinois, Champaign, IL 61820.

We have investigated the possibility that eye position influences the visual responsiveness of neurons in cat area 17. With their heads fixed and direction of gaze monitored via the scleral search coil technique, cats were trained to maintain fixation on a laser beam projected onto a rear-projection screen. When a single neuron was isolated, a bar of optimal size and direction was swept through the retinotopically defined receptive field while the position of the fixation point was varied. The bar was never of behavioral relevance to the animal. For 7 of 40 neurons tested, the visual response evoked by stimuli moving through the receptive field varied with direction of gaze by a least a factor of two. Effects on response magnitude were mainly observed for horizontal gaze shifts. For 6 of the 7 neurons, the larger response was obtained when gaze was directed away from the hemisphere containing the cell. Eye position affected general excitability, since for some cells spontaneous activity also varied as a function of direction of gaze. These gaze-related responses may serve to construct a head-centered frame of reference. (Supported by NIH Grants EY06818 and EY02695).

419.8

SEGREGATION OF SPATIOTEMPORAL RESPONSE PROPERTIES BETWEEN CAT VISUAL CORTICAL AREAS: A 2-DG DEMONSTRATION. **D. Kaufman, J. Greenberg and M. Reivich, U. of Penn. Med. Sch. Phila., PA.19104**

Strong evidence supports the concept that Areas 17 and 18 of cat visual cortex perform qualitatively similar neuronal operations at different spatiotemporal scales (Movshon, Thompson and Tolhurst J. Physiol. '78c, McLean and Palmer, ARVO '88). Recent evidence suggests that PMLS and PLLS contain unit populations with partially non-overlapping spatiotemporal response optima (Zumbroich and Blakemore J. Neurosci.'87). In order to demonstrate the known spatiotemporal segregation between the Area 17 and 18 population at the whole-cortical representation level, and to extend this analysis to the LS cortices, we performed standard C-14 2-DG autoradiography following stimulation in a split-field paradigm. The left visual field contained sinusoidal gratings drifting at 50 deg./sec. (High-velocity) which were presented, at random, from a stimulus list of gratings ranging from 0.1 to 0.5 cycles/deg. The right visual field contained gratings drifting at 1 deg./sec from a list ranging from 0.6 to 4.0 cpd. These spatial frequency ranges were selected to fit the Movshon '78c data for non-overlap of Area 17 vs. 18 spatial frequency response ranges. Each grating's temporal frequency coefficent was selected via Speed = Temp. Freq./ Spat. Freq. so as to maintain constant high (or low) velocity. All gratings were binocularly presented (convergence via a Risley prism), of high contrast, achromatic, of horizontal orientation (to reduce non-convergence artifact) and were drifted either up or down (at random) for 10 sec intervals during the 45 min. 2-DG infusion period. The resulting coronal autoradiograms showed orientation columns in Area 17 of the left hemisphere (Low-velocity stimulated) but no detectable columnar labelling in right Area17. Area 18 of the right hemisphere (Hi-veloc. stim.) displayed orientation columns while left hemisphere Area 18 and Lat. Suprasylvian cortex (bilaterally) showed no detectable columnar label. In other studies, the gratings were all drifted unidirectionally. In these cases no detectable columnar labelling was found in striate cortex. AMLS, and to a lesser extent PMLS, in the left (Low-veloc. stim.) hemisphere, demonstrated wide (~0.5 mm.) columns, supporting physiologic evidence for orientation (Hubel and Wiesel, J. Physiol., '69) and / or directional columns (Sherk , Soc. Neurosci.'88) in L.S. cortex. Presumably, a >40% reduction in the response of the striate population to unidirectional gratings underlies the failure to detect orientation columns in the latter cases.

419.9

RESPONSIVENESS OF CELLS IN AREA 18 AFTER LOCAL INACTIVATION OF AREA 17 IN CATS. Y. Michaud*, C. Casanova, P.A McKinley, S. Molotchnikoff. Université de Montréal, Département de Sciences Biologiques et Centre de Recherche en Sciences Neurologiques. Montréal, Canada, H3C 3J7.

It is well known that area 17 or striate cortex projects to other cortical areas, such as area 18 or V2. However, very little is known on the functional roles of these forward connections. We have studied the effects of a local reversible inactivation of area 17 on the responsiveness of cells in the second visual area (V2). Experiments were carried out on anesthetized and paralyzed adult cats. Tungsten-in-glass microelectrodes were used to record extracellular single-spike activity in V2. An injecting-recording micropipette filled with a stained lidocaine solution was placed in a retinotopically corresponding region in the upper layers of the striate cortex. So far, a total of 20 cells were tested. Out of these, 11 and 9 units were classified as simple-like and complex-like cells, respectively. Our results indicate that the response properties of two-third of the complex cells were modified after the blockade of area 17. In a few cases (2), responses were totally abolished. On the other hand, receptive field properties of only one-third of the simple cells were modified by the inactivation. These preliminary results suggest that the disruption of the feedforward connections is more likely to affect the complex cells of the second visual area.

(supp. CRSNG and FCAR to SM; MRC to CC)

419.10

DIRECTIONAL SELECTIVITY OF CORTICAL CELLS AND THE PRESENCE OF A CONDITIONING STIMULUS - S. Molotchnikoff, C. Morin * (Spon: M. Ptito), Dept. Sci. biol. et Cent. Rech. Sci. Neurol. Université de Montréal (Québec) Canada H3C 3J7.

Neurons of the visual cortex possess fine tuned trigger features which evoke optimal responses. These properties can be modified if a second stimulus (S2) is introduced outside the classical receptive field (CRF) boundaries.This study was aimed at analyzing the responses to a moving light bar when a second or a conditioning slit was also present. Rabbits were anesthetized and prepared for single cell recordings. The first, or test stimulus (S1) was a light slit moving back and forth at the optimal orientation in the receptive field. S2 moved in phase with S1. When presented in isolation, S2 had no effect on spontaneous activity of the cell. But, when the stimuli were applied simultaneously, responses to S1 were significantly altered (modifications > to 25%). Test responses may be increased ($X = 62,6 \pm 28.3$) or decreased ($X = 68,8 \pm 21.9$) $N = 46$. Mobile conditioning stimuli had a more pronounced influence than stationary stimuli (68.8% VS 45.3% $P < .005$, $N = 25$). As the index of directionality increased, the alterations became stronger, and the prefered direction was most strongly affected. The simple cells ($N = 6$) and the end stopping units ($N = 3$) were the most influenced while only 36% ($N = 19$) of complex units reacted to S2. These results indicate that movement sensitivity may strongly depend upon the visual background.

419.11

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

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

In order to assess color sensitivity in cat cortical neurons, we used two types of large background stimuli (50 x 50 degree visual angle) that consisted of a monochromatic and a polychromatic computer-generated noise process of identical luminance superimposed on a moving bar of light. As a rule, we measured the modulatory effect of the background onto the response of the cell to the moving bar. Background stimuli were moved at different speeds inphase or antiphase to the bar. In addition, we used on-off stimuli, which were flickered inphase to the motion of the background.

The most sensitive parameter to changes of spectral composition was directional selectivity that was effected in 80 to 90 % of all cells tested. In contrast, visual latencies, orientation selectivity, and RF-width turned out to be rather robust. Additionally, if the cells responded with excitation to the moving background alone (classical texture response), the spectral composition revealed differential responsiveness in 70 % of the neurons tested.

Under the described stimulus conditions, a very large portion of cat cortical neurons are sensitive to the spectral composition of the stimulus. However, we found no indication for any kind of general preferrence of the monochromatic over the polychromatic stimuli, or vice versa.

Supported by Deutsche Forschungsgemeinschaft

419.12

PROCESSING OF ORIENTATION IN V1 OF THE AWAKE MONKEY. **Y.Trotter*, S.J. Thorpe*, S.Celebrini*, A. Pouget* & M. Imbert* (SPON: J. Servière). Institut des Neurosciences CNRS, Univ. Pierre et Marie Curie, Paris, France.**

It has been suggested that competitive inhibitory interactions between orientation-tuned cortical neurons may be used to increase their orientation selectivity. One prediction of such models is that the orientation selectivity of the initial neuronal response should be relatively low, but that selectivity should increase over time, a suggestion supported by recent data from anesthetized, paralyzed cats (Best et al, in Neural Networks from Models to Applications, I.D.S.E.T, 1989). We have further tested this hypothesis by analysing the responses of neurons in area V1 of the awake primate. Stationary or drifting square-wave gratings, 7° in diameter were presented foveally for 1 second while the animal was performing a fixation task, and the responses of 102 neurons analysed. Eighty-six had visual responses, typically with latencies in the range 40 to 85 ms (mean 58.5 ms) although some had longer latencies (up to 150 ms). Approximately 75% of these responses were orientation selective. We analysed in detail the responses of 24 neurons for which at least five repetitions of all 16 test orientations were available. Mean activity during fixation was about 7 spikes/s. Within 10 to 20 ms of the start of the response, mean activity increased to about 100 spikes/s for the optimal orientation, while decreasing to only 2 spikes/s for the non-optimal one. Indeed, the largest difference between the responses to the optimal and non-optimal stimuli was seen after 10 to 20 ms. We were also able to estimate the bandwidth at half-height which had an average value of approximately 45° (range 15° to 72°). For many cells, this bandwidth was as small at the start of the response as it was later on. It is unclear why our results differ from those of Best et al, but the difference could be due to a species difference, or an effect of anesthesia. However, our results indicate that orientation selectivity can be generated without the use of feedback loops and probably does not require competitive interactions between orientation selective units.

419.13

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

Input to striate cortex of primates consists of two distinct streams: Magnocellular (M) and Parvocellular (P). Whether the M and P streams retain their separate identities or are combined in the striate cortex is a matter of current interest. We sought to answer two questions: 1) Is there a difference in contrast sensitivity between cells in cytochrome oxidase blobs and interblobs? 2) To what extent does contrast sensitivity vary within each (blob/interblob) population?

Using tangential penetrations through striate layers 2/3 in anesthetized and paralyzed monkeys, we measured the response of single cells to drifting sinusoidal gratings at several contrasts, at optimal orientation and spatial frequency. The cortex was stained for cytochrome oxidase and the electrode tracks reconstructed. Contrast gain can be used to reliably differentiate M cells (high gain) from P cells (low gain). However, since cortical cells exhibit thresholds while their subcortical inputs do not, a more complete measure was needed. We therefore derived detection probability *vs* contrast for each cell using ROC analysis, and determined threshold, slope and maximum detection probability from this function.

Our results to date show a remarkable variability in contrast detection across layers 2/3, with no obvious separation between blob and interblob populations.

Supported by EY4888 and NIH GMO 7739.

419.14

STRUCTURAL MODELING OF NONLINEAR NETWORKS IN V1 OF THE MACAQUE MONKEY. J.P. Gaska*, L.D. Jacobson*, H-W Chen*, and D.A. Pollen. (SPON: D. Chad). Department of Neurology, University of Massachusetts Medical School, Worcester, MA 01655.

We have used 2-D spatial/1-D temporal N-ary white noise to visually stimulate cortical cells in V1 of the Macaque monkey. Stimulus-response cross correlation functions were then computed using the methods of Lee & Schetzen (Int. J. Contr. 2:237-254,1965). The resulting (three-dimensional) first order and (six-dimensional) second order cross correlation functions have been used to visualize the detailed space-time structure of interaction within the receptive fields of V1 cells. Examples of simple, complex, and unoriented cells will be shown using dynamic and static visual displays. Methods will be presented that employ such cross-correlation results to evaluate and parametrize nonlinear structural models of multi-input visual neural networks. Supported by NIH grant EY05156 and Air Force grant AFOSR-89-0247.

419.15

FUNCTIONAL CONNECTIVITY BETWEEN VISUAL CORTICAL NEURONS STUDIED BY CROSS-CORRELATION ANALYSIS. Y. Hata*, T. Tsumoto and H. Tamura*. Dept. Neurophysiol., Biomed. Res. Ctr., Osaka Univ. Med. Sch., Kitaku, Osaka, 530 Japan.

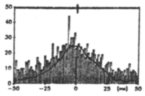
Neurons in the visual cortex are arranged in columnar organization according to their orientation and ocular preferences. However, little is known about functional neural connectivity through which visual information is processed between columns except a report on layer II/III (Ts'o et al. 1986). Present experiments were designed to study functional connectivity of neurons in the horizontal direction in layers II-VI of the striate cortex of cats anesthetized with N_2O in addition to halothane when necessary. Spike discharges were recorded simultaneously from a pair of cells that were separated horizontally by less than 1 mm in the cortex and interactions between them were studied by cross-correlation analysis. Many of the correlated firings showed an existence of common inputs to cell pairs separated by less than 500 μm. In this range of horizontal separation, correlated firings were observed mostly between cells located in the same layer. Also, the correlated firings were found mostly in cell pairs with similar orientation preferences in all the layers including layer IV, which receives inputs mainly from the lateral geniculate nucleus and layer VI. These results suggest that cortical cells which locate in the same layer and have similar orientation preferences tend to share the same input beyond orientation columns.

419.16

FUNCTIONAL CONNECTIVITY REVEALED IN AND OUTSIDE OF RECEPTIVE FIELD OVERLAP BY 3 CROSS-CORRELATION TECHNIQUES. J. I. Nelson[1], M.H.J. Munk*[1], J. Bullier[2] R. Eckhorn*[1]. [1]Biophysics, Philipps University, Marburg, W. Germany 3550; [2]Exptl. Neuropsychology Lab, INSERM 94, Bron, France 69500.

Recent anatomical information on INTERarea connectivity in the visual system increases the need for functional analysis of coupling between neurons and neural patches. We measured coupling between cell patches in A17 & A18 of cat cortex with several techniques: local field potentials (LFP), spike-triggered multiunit activity (MUA) and spike-spike cross-correlations. Measurements were made only during spontaneous activity.

All measures are most likely to show coupling when receptive fields (RFs) are overlapping. Here we distinguish two broad categories of coupling. Coupling is called diffuse when the MUA exhibits a broad hump 300 ms wide centered around the occurrence of the trigger spike. Coupling is focussed when a correlation peak of about 40 ms width appears in the MUA, the LFP shows a wave of hyperpolarization occurring after the trigger spike, and spike-to-spike cross-correlations are obtained. This is more likely to occur when the RFs are overlapping and orientation preferences are matched or crossed, than when they are oblique to one another. (Fig: A18 MUA correlation w/A17 spike is drawn on spike-spike correlation histogram, A17 = 0ms; X-axis 100 ms.)



Exceptionally, we observed focussed coupling without RF overlap. This result is consonant with a recent report on the lack of visuotopic correspondence of the connections between A17 & A18 (Salin, Bullier, Kennedy, J. comp. Neurol. in press).

Supported by DFG Ec53/4 and European Science Foundation ETP Twinning grants; JIN is a Humboldt Travel Fellow.

419.17

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

In addition to the columnar connections, individual cells project over long distances parallel to the cortical surface (Gilbert and Wiesel, J. Neurosci. 3, 1116, 1983). One source of long range connections are pyramidal cells in layer 5 which project into layer 6. A possible role for this connection in generating the long receptive fields of layer 6 cells was suggested by inactivation experiments (Bolz and Gilbert, Eur. J. Neurosci., in press). These experiments demonstrated that local inactivation of layer 5 abolished length summation of layer 6 cells over the portion of their receptive fields corresponding to the blocked region of layer 5. This method, however, does not allow to study the presynaptic side of this connection.

We therefore used cross-correlation analysis to examine both, the pre- and postsynaptic cells involved in the layer 5 to layer 6 projection. Single units were recorded with an electrode advanced in layer 5 and a second electrode, horizontally separated by 0.5 - 3.5 mm from the first electrode, placed in layer 6. So far we obtained 34 peaked correlograms out of 109 cell pairs analysed. Our results reveal the specific nature of this particular cortical connection:

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

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

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

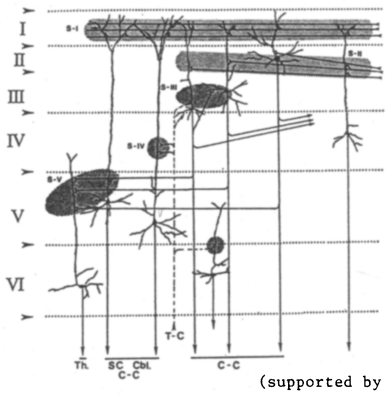
419.18

RECONSTRUCTION OF LOCAL NEURONAL INTERACTIONS WITHIN SINGLE COLUMNS OF THE CAT CEREBRAL CORTEX. S. Reinis and D.S. Weiss. Department of Psychology, University of Waterloo, Waterloo, ONT., Canada.

Adult cats were immobilized with *Pavulon* and anesthetized with nitrous oxide and a long lasting local anesthetic *Marcaine*. Neuronal activity was recorded from Area 18 of the visual cortex using tungsten microelectrodes (1 to 2 Mohms). The electrical potentials were preamplified so that the maximum peak in the record (*leading cell*) was standardized at 1.0 V. Impulse energy below 0.375 V was defined as *mass firing* while all spike activity below 0.125 V was considered noise. Histograms of all interspike intervals within the multiple unit activity recording were calculated. These histograms were called *mass correlograms* when the input data consisted of the *mass firings*. The visual stimulus consisted of a moving light bar which was projected 81 cm in front of the animal. The *mass correlograms* recorded when the visual stimulus was presented along the axis of maximal directional preference contained slightly damped oscillations within the first 200 msec. This result indicated that the movement of a visual stimulus across the common receptive field of the system was accompanied by a circulation of nerve impulses between the participating units. Additional correlograms calculated between *mass firings* and *leading cell* activities showed significant peaks. The functional interactions within the complete neuronal system were reconstructed so that the most common intervals (which formed the peaks of the correlograms) were attributed to real pairs of spikes within the original multiple recording. Computer-generated graphs showed repeated interactions between the neurons which lasted from 1 to at least 50 msec. These interactions were both serial and parallel. The bootstrap procedure was used to verify that the neuronal systems presented in the *mass correlograms* and visual displays were not constructed from a random source of impulse energy. The position of all spikes within each 5 sec record was randomized. *Mass correlograms* were calculated from the randomized set. The bootstrap was replicated 100 times. In no instance did the results from the randomized data form continuous systems as evidenced by the original data.

419.19

MAJOR EXCITATORY PATHWAYS IN THE ANESTHETIZED, SLICED AND AWAKE RAT VISUAL CORTEX, REVEALED BY CURRENT SOURCE DENSITY ANALYSIS, DERIVED FROM THE LAMINAR PATTERN OF EVOKED POTENTIALS TO STROBOSCOPIC AND ELECTRICAL STIMULATION OF WHITE AND GRAY MATTER. G. Vaknin and T.J. Teyler, Neurobiology Dept. NEOUCOM, Rootstown, OH 44272



(supported by EPA and ONR)

419.20

EVIDENCE THAT GLUTAMATE IS THE MAJOR EXCITATORY TRANSMITTER IN CALLOSAL PROJECTIONS TO RAT VISUAL CORTEX. R. L. Berry, A. V. Nowicky, and T. J. Teyler, Dept. of Neuro., NEOUCOM, Rootstown, OH 44272.

To study the electrophysiology and pharmacology of rat visual callosal projections our laboratory has developed a slice preparation which preserves callosal fibers afferent to visual cortical areas OC1 (area 17) and OC2M (medial area 18). In the present study stimulation of white matter (700 to 2500 um medial to recording tracks) yielded field potential profiles recorded with 100 um spacing in tracks perpendicular to the cortex. Recordings were collected before, during and after perfusion with media containing 1 mM kynurenic acid (KYN)--a glutamate receptor blocker. Current source density analysis of field potential profiles allowed spatial localization of current sinks which were integrated to provide measures of excitatory synaptic activity.

Stimulation of callosal fibers yielded major current sinks in OC1 which were usually confined to supragranular layers. Perfusion of media containing KYN resulted in an 80-90% reduction in the area of these current sinks. Callosal stimulation resulted in large current sinks in supra- and infragranular layers in OC2. A 90% reduction of supragranular current sink areas and a 70-100% reduction of infragranular sinks followed perfusion of media containing KYN. Washout of KYN resulted in partial to full recovery of currents in all cases.

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

419.21

BETA-ADRENERGIC EFFECTS OF NOREPINEPHRINE STUDIED IN THE RAT VISUAL CORTICAL SLICE. Alexander V. Nowicky, Richard L. Berry, and Timothy J. Teyler Neurobiology Dept. Northeastern Ohio Univ. College of Medicine, Rootstown, OH 44272.

Norepinephrine (NE) modulates neocortical activity. NE reduces the after-hyperpolarization (AHP) responsible for accommodation in cortical pyramdial cells. The actions of NE are via beta-adrenergic postsynaptic receptors. Voltage clamp studies have shown that NE reduces Ca^{++}- and Na^{+}-dependent K^{+} conductances responsible for slow AHPs in cat sensorimotor cortex, (Foehring, et.al., J. Neurophys. 61(2):245). Coronal slices of rat visual cortex (areas Oc1 and Oc2) were prepared. Stable intracellular recordings were obtained from layer V pyramidal cells. NE, 10-100uM, or the beta-agonist,(-)-isoproteronol (ISO, 1-10uM)) were perfused, or applied by pressure ejection (mM conc.) near the recording site. NE and ISO produced small (<5mV) depolarizations or hyperpolarizations of the membrane potential, without accompanying changes in input resistance. However, spike frequency adaptation and slow AHPs were markedly reduced by NE and ISO. 100uM Timolol (beta-antagonist) perfusions prevented these reductions. In order to examine the effects of NE on cortical synaptic activity, laminar profiles of extracellularly recorded field responses to white matter stimulation were also examined. Current Source Density (CSD) analysis indicated that 10uM NE and 5uM ISO produced increases in both supragranular and infragranular sinks. Our results suggest that beta-adrenergic modulation of visual cortical neurons may have both direct and indirect effects on cortical microcircuitry. (supported by grants from EPA and ONR)

419.22

RECEPTIVE FIELDS IN VISUAL CORTEX OF DJUNGARIAN HAMSTER: A DESCRIPTIVE STUDY. F.W.Grasso,spon:Alan P. Jones. Univ. of Massachusetts, Neuroscience and Behavior Program, Amherst, Ma. 01003

To broaden understanding of the comparative functions of cells in the visual cortex, receptive field maps were made from single unit recordings in a new model system: the visual cortex of the hamster, Phedopus sungorus. Maps were made using two methods employed previously in order to compareresults with work in cats and monkeys. 173 units were mapped with moving black hand-held bars against a white tangent screen. These maps were classified as simple(66%), complex(29%) or unclassified (4%). 110 of these units were also mapped using a computer automated system. This system presented a moving or flashing disc of light on a 27 by 27 grid on a crt screen and collected unit responses. These maps were classed disc(4%),Bar(33%),Composite(21%),diffuse (31%) and unclassified (9%). All bar and disc shaped RFs were found also to be simple while composite and diffuse RFs could be either simple or complex. A classification of the RFs based on the two methods organize the RFs in a way that explicity separates linear and non-linear RFs and maintains a diversity of spatial organization. These are: linear 1)Cicular(31%),Bar(20%),Composite(16%) and non-linear 2)Diffuse(18%) and Composite(6%). This classification shows marked differences between cat,monkey and hamster, yet all classes are present in each species. Thus, the scheme suggested by Djungarinans could be employed to examine the relationship between ecology and RF structure.

BIOLOGICAL RHYTHMS AND SLEEP: OTHER III

420.1

CHARACTERIZATION OF SCN AND IGL IN GOLDEN MANTLED GROUND SQUIRREL USING IMMUNOHISTOCHEMISTRY AND CHOLERA-HRP. L. Smale, J. Blanchard*, R.Y. Moore and L.P. Morin. Depts. of Psychiatry and Neurology, SUNY, Stony Brook, NY 11794.

The suprachiasmatic nuclei (SCN) act as a clock controlling circadian rhythms. The intergeniculate leaflet (IGL) of the thalamus provides a major input to the SCN. In this study, we examined the SCN and IGL of S. lateralis a diurnal ground squirrel. Five squirrels were used and immunoreactivity to GFAP, VIP, vasopressin (VP), substance P (SP), l-enkephalin (L-ENK), CRF, oxytocin (OXY), serotonin (5-HT) and neuropeptide Y (NPY) was evaluated as was the retinal input to the SCN and IGL. Cells containing VIP and VP are found in the ventral or dorsomedial SCN. Cells containing L-ENK are seen in the center. Dense terminal plexes for 5-HT and NPY are also evident. In addition, GFAP immunoreactivity is extremely dense. The ground squirrel SCN contains no immunoreactivity for SP, CRF or OXY. The IGL is identified by a group of NPY-containing cells. However, there is also a small group of L-ENK-containing cells in the IGL with a different distribution. SP and 5-HT-containing fibers and terminals are present in the IGL and the ventral LGN. Retinal input to the hypothalamus is restricted to the contralateral SCN. Within the contralateral LGN, retinal terminals partly overlap the regions containing NPY cells. However, there are clearly some clusters of NPY cells in the geniculate that do not receive input from the contralateral eye. (NS 22168)

420.2

THE EFFERENT PROJECTIONS OF THE HAMSTER SCN REVEALED BY PHA-L. N.L. GOODLESS,* R.Y. Moore and L.P. MORIN. Depts. of Psychiatry and Neurology, SUNY Stony Brook, Stony Brook, New York, 11794.

The circadian clock of mammals resides in the SCN, but little is known about the functional relations between the clock and effector systems. We have approached this problem by studying the efferent projections of the hamster SCN as identified with the anterograde tracer, PHA-L. Sections were immunoreacted according to a modified Gerfen and Sawchencko (1984). The injection site as well as filled cell bodies within the SCN were precisely identified using this method. The present results confirm and extend the distribution of projections previously outlined in the rat (Watts, et al 1987, Stephan, et al 1981). The densest terminal field they described was a relatively cell-sparse zone bordered by the SCN, the paraventricular nucleus, the periventricular nucleus, and the anterior hypothalamus. Analysis of hamster material underscores the dense distribution of fibers which travel within the midline, subparaventricular zone of the hypothalamus, and continue on to the paraventricular nucleus of the thalamus. Other sites that are innervated directly by the SCN are confined to the hypothalamus, adjacent areas of the septum and the thalamus. (NS 22168)

420.3

C-*FOS* PROTO-ONCOGENE EXPRESSION IN THE SUPRACHIASMATIC NUCLEUS: IMMUNOHISTOCHEMICAL CHARACTERIZATION. D.J. Earnest, L.A. Trojanczyk, J. Van Lare, H.H. Yeh, and J.A. Olschowka. Dept. of Neurobiology/Anatomy, Univ. of Rochester Sch. of Med., Rochester, NY 14642

In mammals, the generation and light-dark entrainment of circadian rhythms are governed by an internal timekeeping mechanism located in the suprachiasmatic nucleus (SCN). Since the c-*fos* proto-oncogene appears to function in signal transduction within the brain by coupling extracellular stimuli (e.g., signals causing synaptic activation) to intracellular effector pathways, c-*fos* expression may provide a cellular method for assessing the activation of SCN neurons by various stimuli. Consequently, the present study was conducted to characterize the basal expression of c-*fos* proteins in the SCN.

Following exposure to LD 12:12 (lights-on at 0600h), constant light or dark, Long-Evans rats were sacrificed and perfused at 1000h or 2200h. An antiserum raised to the M peptide, corresponding to amino acids 129-153, was obtained from Dr. Iadarola of NIH and brain sections were processed for the immunohistochemical characterization of c-*fos* proteins in the SCN.

C-*fos* protein-like immunoreactivity in the SCN was concentrated within the nuclei of neurons and was characterized by a pattern of immunostaining in which c-*fos* proteins were generally localized in neurons found in the ventrolateral SCN. These results are consistent with other immunohistochemical studies demonstrating that c-*fos* expression in neurons is confined to the cell nucleus and thus provide evidence that our immunostaining is to c-*fos* protein-like molecules. Since neurons exhibiting c-*fos* protein-like immmunoreactivity are localized in a subdivision of the SCN that receives the terminals of visual projections, further analysis is necessary to examine the possibility that c-*fos* expression in the SCN reflects an important signal coupling the electrical activation of neurons by photic stimuli to long-term transcriptional events mediating the photoentrainment of circadian rhythms. Supported by NS-24661(D.E.).

420.4

THE SUPRACHIASMATIC NUCLEUS (SCN) OF THE CHICK: INTRINSIC ANATOMY, ITS EFFERENTS AND AFFERENTS. D.B. Hodges Jr.* and V.M. Cassone. Department of Biology, Texas A&M University, College Station, TX 77843.

Avian circadian systems comprise multiple circadian oscillators and photoreceptors in the pineal gland, eyes and SCN. However, the site, anatomy and function of the SCN remains unresolved. In this study, immunocytochemical localization of GAD, AVP, VIP, Sub.P, 5HT and NPY was determined within the SCN of the chick. These data were correlated with the distribution of the retinohypothalamic tract (RHT) using intraocular cholera toxin-HRP as an anterograde tracer. Finally, SCN efferents and afferents were studied by stereotaxic microinjection of CTHRP into the SCN. As in previous studies in house sparrows and various mammals, the chick SCN contains GAD, VIP, and Sub.P immunoreactive neurons and 5HT and NPY immunoreactive fibers. No AVP-reactive cells could be found. Second, the SCN receives direct RHT input. Finally, major efferent pathwys of the chick SCN project to the contralateral SCN, PVM, LA, EW and ME. Major afferents to the SCN include the eyes of course, LH, PVM and the periventricular organ, which contains 5HT-immunoreactive cells. Together, the immunocytochemical characteristics, the efferents and afferents of the chick SCN are very similar to those previously described for mammals, further supporting a case for homology between the avian and mammalian SCN. The results of these studies will be valuable in the analysis of avian circadian systems physiology. Supported by NSF Grants BNS 85-19660 and 88-96225.

420.5

AChE POSITIVE NEURONS THAT PROJECT TO THE SCN. C.A. Fuller, R. Nayduch, D.M. Murakami. Dept. of Animal Physiology, University of California, Davis, Ca. 95616.

Previous studies have suggested that acetylcholine may play a role in the phase shifting effects of light on circadian rhythms. In addition, single unit recordings have demonstrated that neurons in the SCN are sensitive to cholinergic agonists. This study examines the potential source of cholinergic projection to the SCN.

Small HRP injections were placed into the SCN. Following a 48 hr. survival, time rats were sacrificed with barbituate overdose, transcardially perfused with fixative, and the brain prepared for histology. Coronal sections were reacted for HRP with TMB and counterstained for AChE. Neurons double labelled for HRP and AChE were identified as potential cholinergic neurons that project to the SCN.

Double labelled neurons were located in two neural regions, the diagonal band and the LASCN. Although the septum contains many cholinergic neurons, none of the cells were double labelled. AChE positive neurons in the LASCN were found to have large cell bodies and elongated soma shapes relative to the total population.

These results suggest that the diagonal band and LASCN may be involved in the association between acetylcholine and the phase shifting effects of light.

(Supported by the STRC grant #0167-01)

420.6

PHOTOPERIODIC, SUPRACHIASMATIC AND PARAVENTRICULAR CONTROL OF TESTIS FUNCTION AND VIP, VASOPRESSIN, BETA-ENDORPHIN AND GnRH IMMUNOSTAINING IN SIBERIAN HAMSTERS. E.L. Bittman, T.J. Bartness, and G.J. DeVries, Depts of Zoology and Psychology, Univ. of Massachusetts, Amherst, and Dept of Psychology, Georgia State Univ., Atlanta 30303.

Short days act through the suprachiasmatic and paraventricular nuclei (SCN and PVN) to regress the gonads of Syrian hamsters. In the European hamster, short photoperiods reduce vasopressin (VP) immunoreactivity in lateral septum (LS), medial amygdala (MA), and bed nucleus of the stria terminalis (BST). We examined the interactive effects of photoperiod, SCN and PVN lesions on testicular weight and neuropeptide staining in another photoperiodic species, the Siberian hamster (Phodopus sungorus). Intact (n=13), SCN lesioned (n=24), or PVN lesioned (n=17) hamsters were maintained in long days (LD, 16L:8D) or transferred to short days (SD, 8L:16D) for 10 weeks prior to acrolein perfusion. Short days induced testicular regression (combined testes weights 0.20±0.06g vs. 0.90±0.02g in LD, mean±SEM; $p<0.05$). Hamsters greater than 4 months old were significantly less responsive to photoperiod. Short days reduced the number of VP immunopositive cells in the MA and the VP fiber content of the LS and the BST, the number of GnRH immunopositive cells in the medial preoptic area at the level of the OVLT, and the number of beta endorphin (BE) immunoreactive neurons in the arcuate nucleus ($p<0.05$). SCN and PVN lesions blocked gonadal regression and eliminated all effects of daylength on neuropeptide staining. VIP staining was unaffected by daylength in the SCN, median eminence or amygdala. SCN lesions eliminated VP immunoreactivity in the thalamic paraventricular nucleus and raised the number of VIP-stained cells in the basolateral amygdala regardless of photoperiod. While the SCN and PVN participate in photoperiodic responses of Siberian hamsters, effects of daylength on GnRH, BE and VP neurons may be either causes or effects of gonadal regression. Supported by NSF BNS 86-16935, 88-09799 and NIH DK3524.

420.7

SYNAPTOGENESIS IN THE RAT SUPRACHIASMATIC NUCLEUS - A LIGHT MICROSCOPIC IMMUNOCYTOCHEMICAL SURVEY. K.B.REPKE*, L.K.LAEMLE, F.L.RICE and R.HAWKES*. Depts. of Anatomy, New Jersey Medical School,Newark NJ 07103 and Albany Medical College, Albany,NY 12208, and Laboratory of Neurobiology, Laval Univ.,Quebec GIJ5B3 Canada.

MabQ155, a monoclonal antibody which selectively binds to an integral polypeptide in synaptic vesicle membranes (Hawkes et al,'85), has been used to survey synaptogenesis in the rat suprachiasmatic nucleus using light microscopic immunocytochemistry in 50um frozen sections. The period studied, the day of birth (P0) to postnatal day 10 (P10), overlaps the reported time of arrival of retinal afferents and the development of circadian rhythms.

On the day of birth the SCN appeared as a relatively clear ovoid area in the ventral region of the otherwise highly immunoreactive hypothal amus. Between P0 and P10, presynaptic terminal density in the SCN increased so that the SCN became indistinguishable from the adjacent hypothalamus. Within the SCN, synaptogenesis followed a rostral to caudal and a peripheral to central gradient, and different regions of the nucleus exhibited different rates and different patterns of synaptogenesis.

420.8

LOCALIZATION OF THE CIRCADIAN PACEMAKER WITHIN THE SUPRACHIASMATIC NUCLEI (SCN). T.K. Tcheng, M.U. Gillette and R.A. Prosser. Neural & Behavioral Biology Prog. and Dept. of Physiol. & Biophys., Univ. of Illinois, Urbana, IL 61801.

The mammalian SCN contain a circadian pacemaker that is expressed *in vitro* as a 24 hr oscillation in the ensemble neuronal firing rate. We are attempting to determine the location and organization of the pacemaker within the SCN. Our approach has been to isolate progressively smaller regions of the SCN by microdissection of the hypothalamic brain slice, then look for circadian rhythms of neuronal activity (CRs) using extracellular recording techniques.

Our previous work has shown that a 500 μm coronal slice from 2-5 mo Long-Evans rats, which contains less than the anterior-posterior extent of the SCN, produces a stable CR for at least 3 days *in vitro*. Reducing the slice to within 100 μm of the paired SCN results in an unperturbed CR.

Our current studies further localize the pacemaker. Bisecting the SCN by severing the commissure connecting the two nuclei has no apparent effect on the CR (N=3). Analysis of activity in the natural dorsomedial (DM) and ventrolateral (VL) subdivisions in the intact slice shows that both regions exhibit CRs that peak synchronously at CT 6.9 on day 2 (N=8). Preliminary data suggest that subdividing the bisected SCN into DM and VL halves results in differential effects upon the CRs in these two regions.

420.9

THE ROLE OF SCN DORSAL EFFERENT PROJECTIONS IN THE TIMING OF THE PREOVULATORY SURGE OF FSH IN GOLDEN HAMSTERS. L. L. Badura,[1] C. L. Sisk, and A. A. Nunez, Physiology & Neurobiology, University of Connecticut, Storrs, CT 06269,[1] and Psychology Dept./Neuroscience Program, Michigan State University, East Lansing, MI 48824.

Horizontal knife cuts that interrupt the connections between the suprachiasmatic nuclei (SCN) and the paraventricular nuclei (PVN) prevent the vaginal acyclicity (anestrus) normally induced by short-day exposure in female golden hamsters. The present study extended these findings by examining the timing of the proestrous FSH surge in animals with hypothalamic knife cuts that blocked gonadal responses to photoperiod. Female hamsters received either a knife cut aimed between the SCN and the PVN, or sham surgery, and were housed under either long (16L:8D) or short (6L:18D) photoperiods for 11-12 weeks. A subset of the animals in each group were then fitted with an indwelling jugular cannula and blood samples were taken hourly over a 24-hr period. Plasma levels of FSH were determined by RIA. Both groups of animals in 16L:8D continued to show regular estrous cycles throughout the experiment. The cannulated animals from both groups in this photoperiod showed peak elevations of FSH on the day of proestrus 3-4 hr before lights out. Animals with knife cuts in 6L:18D also continued to show estrous cycles and peak elevations of FSH at a time identical to that displayed by animals in 16L:8D. In contrast, sham-operated anestrous females in 6L:18D showed peak elevations of FSH approximately 4-5 hr after lights out. Therefore, disruption of SCN dorsal efferent projections prevents the inhibitory effects of short days on estrous cyclicity, and preserves the typical long-day timing of the preovulatory FSH surge.

420.10

ISOLATED SUPRACHIASMATIC NUCLEI IN AGGREGATE CELL CULTURE. J. Ding, J. Buggy, and L. Terracio*. Depts. of Physiology and Anatomy, Univ. of South Carolina, Columbia, S.C. 29208.

Suprachiasmatic nuclei (SCN) were microscopically dissected from perinatal rat brain (from E17 to P4), dissociated, and incubated in rotary suspension to reform organotypic cell aggregates. Electrophysiological and electron microscopic analyses demonstrated the viability of these cells in rotary culture for up to 4 weeks. Immunohistochemical analysis of the aggregates with neurofilament, GFAP, and synaptophysin antibodies showed that outgrowth of cell processes and synaptogenesis are comparable to the intact SCN. Peptidergic neurons characteristic of SCN were also identified immunohistochemically in the aggregates: vasopressin, VIP, and somatostatin. However, clustered plexus groupings of these peptidergic neurons were not evident in the aggregates analyzed thus far, suggesting that organization of SCN-like structures may require unspecified growth factors or interactions with a host brain environment. Aggregates maintained in culture survived transplantation to cerebral ventricles and continued to express cytoskeletal markers and neuropeptides. The reaggregated cell culture system may be a good model to study the structural organization and neurotransmitter distribution of small brain nuclei such as SCN. A potential long-range application of these studies would be development of culture conditions suitable for storage, organization, and selection of brain tissues for neural transplants.

420.11

SUPRACHIASMATIC NUCLEUS (SCN) GLUCOSE UTILIZATION IN VITRO: CIRCADIAN RHYTHM AND EFFECTS OF TETRODOTOXIN (TTX).
F.E. Hospod, G.C.Newman and R.Y.Moore. Dept. of Neurology, SUNY at Stony Brook, Stony Brook, N.Y. 11794 (SPON: A. ROSEN).

In this study we demonstrate that the circadian rhythm of SCN glucose utilization previously demonstrated *in vivo* (Schwartz *et al*, J. Comp. Neurol., 1980) continues to be expressed in hypothalamic brain slices containing SCN. The effects of TTX on this rhythm are also described.

Hypothalamic brain slices were isolated from 175g Sprague-Dawley rats maintained in 12:12 L:D schedule for at least 3 weeks. The lights were kept off the day of sacrifice and rats were decapitated in dim red light. Brain slices were pre-incubated at 37°C. in K-R for 90 min, incubated with ^{14}C-2DG (0.2μCi/ml) for 45 min and rinsed for 30 min. Autoradiograms were obtained from cryostat sections and brain slice glucose utilization (SGU) calculated (see Newman *et al*, this meeting) at nine times of the circadian cycle. SGU was also measured at six times in the presence of TTX.

These studies reveal that SCN SGU is lowest at CT17 (lights on CT00), gradually rises from CT00 to a maximum at CT09 and gradually declines until CT15. Values of SGU from CT17 to CT06 are similar to those found *in vivo* but those from CT09 to CT15 are considerably higher. In the presence of TTX, SGU is reduced to about 60% of the values found in the absence of TTX for time points between CT17 and CT06. However, at CT09 and CT12, TTX dramatically reduces SGU to levels similar to those at CT17 with TTX.

The circadian rhythm of SCN glucose utilization persists in hypothalamic brain slices although signficant differences are observed between CT09 and CT15. In the presence of TTX, SCN SGU increases above baseline only between CT00 and CT06. These results suggest that slice isolation eliminates an inhibitory influence active *in vivo* during late subjective day.

420.12

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

The suprachiasmatic nuclei (SCN) contain an endogenous circadian clock that survives in vitro, where it continues to oscillate with a 24 hr period. We have been investigating the possible involvement of cAMP in the biochemistry underlying the SCN clock. We have shown previously that daytime but not nighttime treatments that increase endogenous cAMP levels in vitro phase-shift the SCN clock by up to 5 hr (J.Neurosci.9:1073, 1989). To further elucidate the role of cAMP, we measured in vitro levels of cAMP within the SCN.

Hypothalamic brain slices containing the SCN were prepared during lights-on from adult Long-Evans rats housed in a 12:12 LD cycle. At circadian times (CT) 4, 10, 16 and 22 slices were removed from the slice chamber and rapidly frozen on dry ice. Punches containing the SCN or nearby hypothalamus were removed using a 26 gauge needle and pooled. cAMP levels were assayed by RIA after Steiner, and protein levels were assayed by the method of Bradford.

Significant peaks in cAMP (pmole/mg protein) were found in the SCN at CT 10 (43.56$\pm$ 6.93, N=14) and 22 (50.93$\pm$10.13, N=12), with low levels at CT 4 (30.01$\pm$4.57, N=10) and 16 (20.41$\pm$3.19, N=9) (ANOVA and Scheffe analyses). Hypothalamic cAMP levels showed no clear rhythm. Together with our previous data on phase shifting, these results suggest that cAMP may be an integral component of the SCN circadian clock.

420.13

EFFECTS OF TETRODOTOXIN ON THE CIRCADIAN PACEMAKER IN SUPRACHIASMATIC EXPLANTS *IN VITRO*.
S.M. DiGiorgio*, C.D. Sladek and D.J. Earnest (SPON: I. Shoulson). Dept. of Neurobiology/Anatomy, Univ. of Rochester School of Medicine, Rochester, NY 14642

Consistent with their central role in the generation of circadian rhythms, explanted neurons from the suprachiasmatic nucleus (SCN) release vasopressin (VP) in a circadian fashion *in vitro*. To study the role of Na-generated action potentials in the circadian timekeeping mechanism in the SCN, the present study examined the effect of tetrodotoxin (TTX), a Na-channel blocker, on the rhythm of VP release from perifused SCN explants.

SCN explants were dissected from the hypothalami of male rats and maintained individually in a perifusion culture system. On day 2 in culture, test explants were exposed to TTX for 6, 8 or 12 hrs near the onset of the subjective day or night. VP levels in the perifusate were determined by RIA.

TTX treatment during the subjective day blocked the peak in VP output that normally occurs during this portion of the circadian cycle while treatment during the subjective night had little influence on the basal levels of VP release expressed during this period. Most explants continued to express circadian rhythms in VP release without any sign of perturbation in the cycles subsequent to treatment, regardless of the time of administration. However, circadian timekeeping in some of the explants exposed to TTX during the subjective day was rendered arrhythmic by this treatment, such that VP output remained at basal levels without any sign of variation. This TTX-induced arrhythmicity is not indicative of a chronic disruption of mechanisms of peptide release because KCl exposure stimulated VP release from explants failing to express circadian rhythmicity after TTX treatment. These results further suggest that the expression of circadian rhythmicity by the SCN is dependent on Na-generated action potentials. Furthermore, the arrest of circadian rhythmicity in some TTX-treated explants may reflect the singular behavior of the pacemaker in the SCN in response to the inhibition of electrical impulses at a critical phase of the circadian cycle. Supported by NS-24661(D.E.).

420.14

MELATONIN RESETS THE SUPRACHIASMATIC CIRCADIAN CLOCK IN VITRO.
A.J. McArthur, R.A. Prosser, and M.U. Gillette. Dept. of Physiol. & Biophys. and Neural Biology and Behavior Program, Univ. of Illinois, Urbana IL., 61801.

The pineal hormone melatonin (MEL) has been implicated in circadian control mechanisms both because daily injections of MEL can entrain rodents (Cassone *et al.*, 1986) and the suprachiasmatic nuclei (SCN) have a high density of specific MEL receptors that varies diurnally (Laitinen *et al.*, 1989). We are examining the ability of exogenous MEL to directly reset the phase of the SCN pacemaker *in vitro*.

Coronal hypothalamic slices containing the SCN were prepared from 8 wk old male Long Evans rats housed in a 12L:12D cycle. At circadian time (CT) 2 or 10, the media in the brain slice chamber was replaced for 1 hr with freshly made media containing 10^{-9} M MEL. All measurements of phase were made by monitoring extracellular firing rates and determining the time of peak in the ensemble neuronal activity on day 2 *in vitro*. Phase shifts were determined by comparing this value with the time of peak neuronal activity on day 2 in untreated slices (CT 6.9 $\pm$ 0.2, N=8).

Treatment of the SCN with MEL at CT 10 induced significant advances (ϕ_A) in the time of the peak on day 2 (3.8 $\pm$ 0.1 hr ϕ_A, N=4), whereas the SCN rhythm was unperturbed by MEL applied at CT 2 (0.1 hr ϕ_A, N=2). These results suggest that MEL may play a role in regulating the SCN pacemaker in the late subjective day. (Supported by NINCDS grant NS22155).

INTERHEMISPHERIC RELATIONS

421.1

VISUAL CONTROL OF HAND POSTURES IN INDIVIDUALS WITH CALLOSAL AGENESIS OR CALLOSOTOMY.
M. Lassonde, M.T. Perenin*, M. Goodale, M. Labonté*, and L. Jakobson. Département de Psychologie, Univ. de Montréal, Montréal, Qué. H3C 3J7 and Univ. of Western Ontario, London, Ont. N6A 5C2, Canada.

Transporting a limb to a visual target involves mostly the proximal musculature which receives input from motor cortex in both hemispheres. The relatively independent movements of the hands and fingers, however, depend almost entirely on projection systems from the contralateral hemisphere. Thus, early work by Brinkman and Kuypers (1973) demonstrated that the information necessary for controlling posturing of the hand ipsilateral to the visually activated hemisphere depends on the integrity of the callosal system. The present study examined visual control of hand postures and finger movements in 2 subjects with callosal agenesis and in 2 epileptic patients who had undergone a therapeutic callosotomy. Subjects were submitted to two tasks: 1. reaching toward and placing their hand in a slot on a vertically mounted disk. 2. reaching toward the disk and retrieving a knob inserted in the slot. For some blocks of trials, the disk was located in the visual field ipsilateral to the hand being used, while in others the disk was placed in the contralateral field. Subjects were required to maintain central fixation while reaching. Age- and IQ-matched control subjects performed the tasks with little difficulty using either hand in either visual field. The acallosal and callosotomized subjects, however, were significantly more imprecise and displayed more shaping errors than their controls under all conditions. This was especially marked when the left hand was used and/or when the left visual field was the source of stimulus presentation. The results are discussed in terms of left hemisphere superiority for motor control.

421.2

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

The discrimination and recognition of complex tonal spectra by the left and right hemispheres of two callosotomy patients with bilateral language competence were tested using sound-picture and sound-word matching tasks. Two sets of probes were presented binaurally: 1) pairs of spectra composed of a fundamental and its first five harmonics, the timbres of which differed in the reciprocal intensities of the third and fourth harmonics in 50% of trials; and 2) individual spectra whose timbres were characteristic of different musical instruments. Visual targets were lateralized tachistoscopically and consisted of, respectively: 1) "same" and "different" choices presented simultaneously in alternate quadrants; and 2) instrument line drawings or names, which matched the probe in 50% of trials.

A double dissociation was observed, indicating that acoustic-discriminative and semantic-associative processes were differentially distributed within the right and left hemispheres, respectively. The results argue against a strict verbal/nonverbal dichotomy as the basis of hemispheric specialization in auditory pattern perception and are consistent with the notion that the meaningfulness of nonverbal sounds critically influences laterality effects. The functional basis of the left hemisphere advantage in semantic-associative processing may be a superior capacity for multimodal (auditory-visual-lexical) integration rather than lexical access alone.

Supported by NIH 5P01-NS17778 and Javits Award 1 RO1-NS22626.

421.3

EFFECT OF ADVANCE INFORMATION ON HEMISPHERIC ASYMMETRY FOR MENTAL ROTATION IN A COMMISSUROTOMISED SUBJECT. M.C. Corballis, Dept. of Psychology, Univ. of Auckland, New Zealand, and J. Sergent, Montreal Neurological Inst., Montreal, Que., Canada H3A 2B4.

The objective was to determine if the strong right-hemispheric advantage for mental rotation of letters shown by L.B., a commissurotomized subject, would be influenced by advance information allowing formation of an image of the letter (R) in the to-be-presented orientation. Without this information, RT analysis confirmed the right-hemispheric advantage: estimated rotation rate and overall RT were slower with RVF than with LVF presentation. This pattern was reversed with advance information, suggesting a left-hemispheric advantage in generating the appropriate image. However advance information impaired right-hemispheric performance as much as it benefited left-hemispheric performance.

421.4

HEMISPHERIC ASYMMETRIES IN MEMORY FOR REAL AND SURREAL ART. Dahlia W. Zaidel and Asa Kasher*. Dept of Psychology, UCLA, Los Angeles, CA 90024-1563.

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

Here, the hemispheric status of the way in which two art styles treat reality was investigated with the hemi-field tachistoscopic technique. A series of 24 "surrealistic" vs "realistic" paintings was first inspected briefly by normal subjects and then memory was probed by flashing the original plus decoys in the left (LVF) or right (RVF) visual half-fields. A recognition paradigm was used and a binary choice was made in responding. Results showed a RVF superiority for the surrealistic paintings. There was no hemi-field difference for the realistic paintings. In two additional experiments, 16 of the same paintings were used as targets in conjunction with metaphoric vs literal titles constructed for them. In one experiment, memory for the paintings alone was probed while in the other, memory for the titles alone was probed. A RVF superiority emerged for the metaphoric titles, especially if paired with surrealistic paintings. Taken together, the results suggest a left hemisphere advantage in processing meaningful, yet incongruous arrays, both pictorial and linguistic.

421.5

LATERALITY OF BRAZILIAN ADULTS AND ITS RELATIONSHIP WITH PERFORMANCE IN LEARNING A SECOND LANGUAGE. S.L.Schmidt*, A.A. Hofke* (SPON:R.Lent) I.BIOFISICA UFRJ,I.BIOLOGIA UERJ Rio de Janeiro - Brasil

In spite of the association of left-handedness with neurological deficits, there is little empirical evidence for performance differences between left-handers and right-handers. We described the distribution of laterality in adult Brazilians and looked for its relation with performance to learn a foreign language. The distribution of handedness of 1,017 Portuguese-speaking Brazilian students undertaking of English or German courses was evaluated using the abridged form of the Edinburgh Inventory. Information about handwriting posture, instruction level and foot preference were also obtained. Performance in learning a foreign language was evaluated by a standardized grade attributed to each student in three exams. Results revealed that the distribution of the laterality scores is J-shaped. Inverted handwriting posture was found to be more frequent among left-handers (40%) than among right-handers (5%). The number of dextral females (92%) was found to be greater than that of males (89%). An analysis of variance for handedness, sex, instruction level, and age showed a significant effect of handedness on the standardized grade. Strong right-handers have a better performance than weak right-handers. These results suggest that not only left-handers but also right-handers represent a heterogeneous group.

421.6

STUTTERING AND VOLUNTARY DIFFERENTIAL HEMISPHERIC ACTIVATION STUDIED WITH COMPUTERIZED EEG AND SPECT. P.S. Gott, C.M. DeGiorgio,* D.C.P. Chen* and E.C. Hughes*. Depts. of Neurology, Nuclear Medicine, and Otolaryngology, Univ. of Southern California Sch. of Med., Los Angeles, CA 90033.

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

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

421.7

VISUAL EVOKED POTENTIAL ESTIMATES OF INTERHEMISPHERIC TRANSFER TIME IN HUMANS: EFFECTS OF VERTICAL POSITION WITHIN A HEMIFIELD. C. D. Saron*, R. J. Davidson* and J. Ellwanger* (SPON: P. Spear). Dept. of Psychology, University of Wisconsin, Madison, WI 53706.

Studies in Macaques have demonstrated asymmetries in callosal projections in dorsal compared with ventral visual cortex. This study examined whether visual evoked potential (VEP) estimates of interhemispheric transfer time (IHTT) might reflect such differences in humans.

Hemifield (LVF or RVF) checkerboards (29 cd/m^2, 85% contrast ratio, 4.0°V x 2.9°H, 1° checks, ISI=2-5 s, duration=10 ms) in each of three vertical positions (Lower (L), 10° Horizontal (H) and Upper (U)) with equal eccentricity (5.1° to mid-nasial edge) were presented in blocks of 100 per location to 18, 18-24 yr. old, right-handed males. L and U stimuli began 1.7° from the horizontal meridian. Ss lifted both index fingers in response to each stimulus.

VEPs were recorded from lateral occipital sites between O1/O2 and T5/T6 referred to linked ears. Estimates of IHTT were computed for P100 and N160 components by subtracting peak latency contralateral to the stimuli from the corresponding peak latency from the ipsilateral site.

Mean(sd) P100 IHTT estimates (in ms) and the percent of subjects showing effects in the anatomically predicted direction were: LVF-L=12(20), 69%; RVF-L=17(12), 92%; LVF-H=17(12), 100%; RVF-H=22(13), 100%; LVF-U=10(12), 76%; RVF-U=16(16), 93%. N160 IHTT estimates were: LVF-L=11(13), 83%; RVF-L=24(21), 91%; LVF-H=19(18), 94%; RVF-H=24(16), 100%; LVF-U=5(22), 61%; RVF-U=19(24), 73%.

The H conditions resulted in the greatest % of subjects with IHTTs in the anatomically predicted direction. The increased percentage of negative IHTTs in the L and U conditions makes interpretation of these results in terms of underlying callosal connectivity problematic. However, a main effect for Vertical Position on N160 latency for contra-, but not ipsilateral values suggests that the transferred response may be less sensitive to location.

421.8

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

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

A tachistoscopic syllable identification task was given to right-handed men and women (n = 64). Half viewed nonsense syllables in the LVF, RVF, and CVF (center, at fixation). The other half viewed them in the LVF, RVF, and BVF (bilateral redundant). Error patterns for LVF and RVF syllables differ, and CVF and BVF error patterns are intermediate to patterns in the unilateral fields. Therefore, subjects appear to rely on both hemispheres to process CVF and BVF syllables, rather than using only the language-specialized left hemisphere. Individual differences in this pattern may reflect differences in the degree to which subjects respond to a syllable task with differential arousal of the left hemisphere.

In addition, accuracy for identifying LVF and RVF syllables was strongly affected by the lateralization of the previous stimulus. Performance in the RVF was better following a LVF trial than following a RVF trial. In contrast, performance in the LVF was worse following a LVF trial than it was following a RVF trial. Effects of CVF or BVF trials on subsequent performance in the two unilateral fields were like those of LVF trials. These results suggest that the visual field of stimulus presentation has an effect on the pattern of arousal of the cerebral hemispheres.

421.9

STRIATE, PARASTRIATE AND PERISTRIATE LEFT-RIGHT VOLUMETRIC ASYMMETRIES IN NEONATAL AND JUVENILE HUMAN BRAINS. K.S. Pollan*, L.W. McCallister, D.J. Woodward, M-C de Lacoste (SPON: R. Lebovitz). *Dept. Cell Biology and Anatomy, U.T. Southwestern Medical Center, Dallas, TX 75235.*

We have previously reported significant left-right regional volumetric asymmetries in striate (ST) and extrastriate (XT) visual areas of the human brain (Soc. Neurosci., 1988; de Lacoste et al). The aim of this study was to determine if XT asymmetries in the neonatal and juvenile human are more pronounced in parastriate (PARA) or peristriate (PERI) cortex.

Neonatal and juvenile (n=3) occipital lobes were sectioned bilaterally in complete series at 50μm in the coronal plane with a large-stage freezing microtome. Adjacent mounted sections at 5mm intervals were processed using a basic Nissl and the Gallyas silver stain for cyto- and myeloarchitectural differentiation of ST, PARA and PERI visual areas. Nissl-stained sections were then videodigitized and the CARP software (Biographics, Inc.) was utilized for semi-automated 1) delineation of the external and internal boundaries of cortical gray and 2) computation of sectional areas. Regional volumes for ST, PARA and PERI cortex and left-right indices of asymmetry were calculated using previously described algorithms (de Lacoste et al., 1988).

Preliminary results indicate that in the neonatal and juvenile human brain, both ST and XT visual areas evidence left-right volumetric asymmetries. However, within XT cortex regional volumetric asymmetries of PERI or association cortex are several-fold more pronounced than those of PARA or belt cortex. The functional significance of asymmetry in visual association cortex remains to be determined.

Supported by HD21711 to MCL and the Biological Humanics Foundation.

421.10

ACCELERATED RECOVERY FROM NEGLECT AND BEHAVIORAL ASYMMETRIES IN RATS WITH MEDIAL AGRANULAR PREFRONTAL CORTEX LESIONS. M.R. Smith, J.M. Vargo, and J.V. Corwin. Dept. of Psych., Univ. of New Orleans, New Orleans, LA 70148.

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

Rat subjects (Ss) received aspiration lesions of either the left AGm (L-AGm, n=25) or the right AGm (R-AGm, n=16), and 3h later were tested for neglect (orientation deficits to visual, auditory, and tactile stimuli). Each Ss was again tested for neglect immediately following the environmental manipulation. In addition to the orientation test, slope and open field tests were conducted 3 times/week for 3 weeks.

The major statistically significant findings are: 1) lateralized differences in general responsivity were shown by 3h postsurgery; 2) L-AGm Ss displayed accelerated recovery of function following 48h exposure to light deprivation only; and 3) L-AGm Ss showed an ipsilesional turning preference, whereas R-AGm Ss showed no preference. These results support and extend earlier findings on laterality and recovery of function following AGm lesions. Supported by grant NS 24975 from NINCDS to J.V.C.

421.11

LATERALIZATION OF CONDITIONING-RELATED PROCESSES IN NORMAL RATS. R. Tomer, A. Laferriere, L. Burger and A. Robertson. Dept. Psychology, University of Alberta, Edmonton, AB, T6G2E9.

To determine whether functional assymmetries related to attentional phenomena could be shown in normal rats, forty two rats were given a set of tests in order to determine the presence of consistent side biases in sensorimotor responsiveness. Between 35 and 50 percent of rats displayed consistent and persistent side biases. 19 of the most lateralized rats were then conditioned by having a signal light come on before and during brief dipper presentations. One group received light presentations on the preferred side, the other on the neglected side. Rats receiving the light on their preferred side displayed evidence of having acquired a conditioned approach response to the light. Rats having received light presentations on the neglected side failed to display signs of effective conditioning. This suggests that, in a proportion of normal rats, some processes related to stimulus-reward conditioning are subject to lateralization. These effects could be useful for the study of assymmetry and its relation to psychopathology.

421.12

TESTOSTERONE AFFECTS SOME MEASURES OF RAT BEHAVIORAL ASYMMETRY. K.J. Schultz. Psychology Dept., Univ. of Winnipeg, Winnipeg, Manitoba, Canada, R3B 2E9.

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

421.13

SPONGIFORM DEGENERATION IN A HIGHLY LATERALIZED MOUSE LINE G.F. Sherman, R.L. Collins, V.H. Denenberg, D.M. Press*, and A.M. Galaburda. Depart. of Neurol., Beth Israel Hospital and Harvard Med. School, Boston, Ma., 02215, Biobehavioral Sciences Graduate Program, Univ. of Ct., Storrs, Ct. 06268, and The Jackson Laboratory, Bar Harbor, Me., 04609.

Developmental brain pathology is associated with deviations of cortical asymmetry and anomalous handedness. In the present study we looked for evidence of CNS neuropathology in two lines of mice bred for either strong (HI) or weak (LO) paw preference and mice from the HET progenitor population [Collins, in S.D. Glick (ed.), *Cerebral Lateralization in Nonhuman Species*, Academic Press, 41-70, 1985].

Eighteen HI (9 male, 9 female), 16 LO (8 male, 8 female), and 18 HET (8 male, 10 female) adult mice were used in this study. Brains were embedded in celloidin, coronally sectioned at 35μm, and stained with cresyl violet. The sections were examined under the light microscope and the presence, type, and location of the abnormalities were recorded.

Surprisingly, 12 (67%) of the HI mice had severe spongiform degeneration involving neocortex, hippocampus, septum, and hypothalamus. The distribution and severity of the degeneration varied among animals, although all animals were affected bilaterally. The neocortex was the most severely affected structure. Layers V and VI contained the greatest density of vacuoles, although all cortical layers were affected. Eight (88%) females and four (44%) males from the HI line were affected, while only 1 male and no females from the LO line showed spongiform changes, and one male and two females from the HET group had a few vacuoles in the hippocampus and septum, but not the neocortex. In addition, ectopic neurons were seen in layer I of the cortex in 3 mice from the LO line and 1 mouse from the HI line. The ectopias were seen in mice with and without spongiform degeneration.

This work was supported, in part, by NIH grant 20806 and by the Orton Dyslexia Society.

421.14

INTENSITY OF APOMORPHINE-INDUCED CIRCLING IS GREATER FOLLOWING LEFT NIGROSTRIATAL (6-OHDA) LESIONS THAN RIGHT-SIDED LESIONS. R. Sullivan*, M.G. Ziegler and H. Szechtman (SPON: J.M. Cleghorn) Dept. Biomedical Sci., McMaster Univ., Hamilton, Ontario, CANADA L8N 3Z5.

Circling in response to apomorphine (APO) was studied in rats with left or right nigrostriatal lesions (6-OHDA), who had been evaluated for directional bias prior to lesioning. Two weeks post-lesion, 21 male Wistar rats were injected with APO (0.25 mg/kg, sc) and placed in an automated rotometer. Rats performing more than 30 net contraversive full turns during the 15 min. test (19 of 21 rats) were compared statistically. Animals with left-sided lesions (N=9) were found to perform significantly more net contraversive turns (mean=120.6 circles) than those with right-sided lesions (N=10, mean=91.7; $p<0.05$, one-tailed t-test). Re-examination of earlier data of APO-induced circling in the rotometer, confirmed this side of lesion effect ($p<0.05$, one-tailed t-test). Combining the two sets of data (N=58) demonstrated a robust significant difference ($p<0.02$, one-tailed t-test). Pre-lesion measures of directional preference, based on the predominant direction of swimming in a 2m-diameter pool, were found to be unrelated to post-lesion circling. The data are consistent with left striatal dominance in the population of male rats, an asymmetry which may override individual directional preferences. [Supported by NSERC. HS is a Research Assoc. of OMHF].

421.15

DO CALLOSALLY PROJECTING NONPYRAMIDAL NEURONS EXIST IN RAT VISUAL CORTEX ? C. Hughes and A. Peters. Dept. of Anatomy, Boston Univ. School of Med., Boston, MA 02118.

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

421.16

ROLE OF MIDBRAIN IN INTERHEMISPHERIC TRANSFER IN CATS. J. M. Sprague and A. C. Rosenquist. Dept. of Anatomy, Univ. of Pennsylvania Sch. of Med., Phila., PA 19104-6058.

Contralateral hemianopia follows unilateral removal of all visual areas in occipital-parietal-temporal cortex. Orienting responses to stimuli in the "blind" visual field are restored by section of the tectal commissure. (Sprague '66; Sherman '74; Wallace et al. '87, '89). Midbrain commissurotomy also enhances cognitive functions. Split-chiasm cats show marked defects in interhemispheric transfer of shape discriminations when measured from intact hemisphere to side with lesion in suprasylvian gyri (Berlucchi et al. '79). (Deficit does not occur to easier discriminations of low frequency gratings.) When collicular commissure is sectioned after cortical lesion, normal transfer of shape discriminations is restored. Animals after suprasylvian lesions do not appear to have either sensory or motor deficits. Therefore it is possible that the deficit in transfer is due to visual neglect stemming from inattention in the lesioned hemisphere. The known role of the superior colliculi in selective attention directed by inhibitory fibers in the commissure, suggest that restoration of transfer after collicular commissurotomy is due to enhancement of attention required to make difficult discriminations. Not yet known is whether this function, like orienting, is related to the nigrotectal projection, part of which passes through the tectal commissure. (Supported by NIH · EY02654 and EY04906).

421.17

THE CORTICAL ORIGIN AND COURSE OF FIBERS IN THE PERIVENTRICULAR WHITE MATTER OF TWO COMPONENTS OF THE SPLENIUM OF THE CORPUS CALLOSUM IN MACAQUES. S. Demeter[1], D.L. Rosene[2], and G.W. Van Hoesen[3]. [1]Dept. of Neurol., Univ. Rochester Med. Ctr., Rochester, NY 14642; [2]Dept. of Anat., Boston Univ. Med. Ctr., Boston, MA 02118; [3]Dept. of Anat., Univ. Iowa Sch. of Med., Iowa City, IA 52242.

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

To determine the sources of these two splenial components, we examined by autoradiography 33 macaques *(M. macaca* and *M. fascicularis)* with small cortical injections of radioactively labeled amino acids in the ventral temporal and occipital lobes. In the temporal lobe the occipitotemporal sulcus formed the boundary between the fields of origin of the two components. The inferior temporal cortex, located lateral to the occipitotemporal sulcus, gave rise to fibers that coursed dorsolaterally around the atrium of the lateral ventricle and slightly rostrally thereto to cross the midline in the dorsal splenium and the caudal part of the body of the corpus callosum. By contrast, the posterior parahippocampal gyrus gave rise to fibers that coursed in the inferior forceps to cross in the ventral splenium.

In the occipital lobe there was a similar topographic division. Fibers that arose dorsal to the calcarine sulcus and in the dorsolateral and ventrolateral occipital lobe entered the forceps major to cross in the dorsal splenium. Fibers that arose between the calcarine and occipitotemporal sulci and slightly lateral to the latter, entered the inferior forceps to cross in the ventral splenium.

BEHAVIORAL PHARMACOLOGY: DOPAMINE

422.1

AN INCREASE IN NEUROLEPTIC CONCENTRATION AMELIORATES HALOPERIDOL INDUCED ABNORMAL LICKING PATTERNS: a necessary characteristic of an animal model for tardive dyskinesia. SJ Wasserman and KM Kantak. Lab. of Beh. Neurosci., Dept. of Psych., Boston Univ., Boston, MA. 02215.

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

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

422.2

HALOPERIDOL (HAL) SLOWS RATS' RESPONDING BY LENGTHENING THE TIME REQUIRED TO SWITCH FROM ONE MOTOR RESPONSE TO ANOTHER: A PARKINSON-LIKE EFFECT. **S.C. Fowler, M.A. Kirkpatrick*, P.D. Skjoldager*, and R.M. Liao*. Depts. of Psychology and Pharmacology, University of Mississippi, University, MS 38677.**

Classical dopamine-receptor-blocking neuroleptics decrease average rate of operant responding by rats in at least two ways: by producing a total cessation of responding before a session ends and by slowing the response itself. In order to extend this work, the effects of low doses of hal (0.04 and 0.08 mg/kg) were assessed in rats trained in operant chambers that permitted recording the time intervals between the successive behaviors occurring within a typical cycle of the operant/consummatory complex. Results showed that two intervals (latency from muzzle entry into the reward well to initial tongue protrusion and latency from muzzle exit to the next forelimb response) were proportionately lengthened more than others. Hal appears to disrupt response selection and initiation processes in rats. These results provide additional evidence for homology between neuroleptics' effects in man and rat. (Supported by MH 43429)

422.3

DRUG DISCRIMINATION: REBOUND FOLLOWING A SINGLE DOSE OF HALOPERIDOL. W.F. Caul, J.R. Jones, & R.J. Barrett. Dept. of Psychology, Vanderbilt University, Nashville, TN 37240

Barrett and Steranka (1983) reported that 24 hr after a chronic regimen of 10 consecutive daily injections of 1 mg/kg haloperidol (HD) animals previously trained to discriminate between amphetamine (AM) and saline responded as if they had been injected with a small dose of AM. The experiments reported here were designed to assess whether a similar rebound phenomenon would result from a single dose of HD. Rats were trained to discriminate .5 mg/kg AM from distilled water (DW) (cf. Caul, Burgen, and Barrett, 1989). Four groups (n=8) were formed to allow testing of the drug's effect at 12, 18, 24, and 36 hr post-injection. Each animal was given 0, .5, 1.0, and 1.5 mg/kg HD at its appropriate injection time in a counterbalanced fashion with one week between each test. Although predicted patterns were observed, no reliable differences were found. Four weeks later, half of the animals were injected with 1 mg/kg HD 24 hr prior to testing, whereas the others were given DW. When tested, the HD group responded 36% of the time on the AM-correct lever, whereas the DW group responded at 28%. The failure to find significant temporal patterns of post-HD rebound phenomena using a within-subjects design and the observation of rebound in the between-subjects study have both theoretical and methodological importance.

422.4

BEHAVIORAL AND BIOCHEMICAL EFFECTS OF PERTUSSIS TOXIN ON DOPAMINE RECEPTORS IN VIVO. C. Marin, S.A. Parashos, and T.N. Chase. ETB, NINDS, NIH, Bethesda, Md 20892.

Dopamine receptors have been classified in two subtypes: the D-1, positively coupled to adenylate cyclase by the stimulatory Gs protein and the D-2, either not coupled or negatively coupled to the enzyme by the inhibitory Gi protein. Behavioral experiments suggest important functional interactions between D-1 and D-2 receptors, with the D-1 system normally acting to increase motor activity induced by stimulation of D-2 receptors. In this study D-1/D-2 receptor interactions were evaluated in rats by intrastriatal administration of pertussis toxin (PT), which inhibits the Gi protein linked to D-2 receptors. After unilateral injection of 1.5 ug, the animals developed a marked postural asymmetry towards the injected side. The selective D-2 agonist quinpirole (1 mg/kg, ip) produced significant ipsilateral rotation, while the selective D-1 agonist SKF 38393 (10 mg/kg, ip) had no such effect. Combined treatment with both drugs potentiated the effect of quinpirole alone. Rotation induced by quinpirole was blocked completely by the selective D-1 antagonist SCH 23390 (0.5 mg/kg, ip) and partially by the selective D-2 antagonist raclopride (1 mg/kg, ip). Treatments which resulted in rotation, quinpirole alone and with SKF 38393, also resulted in asymmetry in striatal cAMP levels. These findings provide support for a relation between D-1/D-2 behavioral interaction and alterations in cAMP levels and indicate an important role for G proteins in dopamine receptor interactions.

422.5

MODULATION OF YAWNING BY STRIATAL DOPAMINE AUTORECEPTOR SENSITIZATION. B.I. Diamond, A. Hitri*, N.A. DeMartines*, H. Nguyen*, E. O'Neal* and R.L. Borison*. Department of Psychiatry, Medical College of Georgia, Augusta, GA 30912.

Yawning is a behavior modulated by Dopamine (DA) D1 and D2 presynaptic autoreceptors. This behavior may be relevant to certain disease states. It was the aim of this study to examine these DA receptors in male Sprague Dawley rates (200 grams) treated chronically with a neuroleptic. Animals had bilateral striatal cannula implanted for injection of either the D1 or D2 agonist SKF38393 or LY171555 and the D1 or D2 antagonist SCH23390 or haloperidol. Fluphenazine (5 mg/kg; i.p.) was injected for a 2 week period. Animals were rated for yawning and stereotyped behaviors in response to apomorphine (0.05 to 0.2 mg/kg; s.c.) three days later. Yawning behavior was inhibited by 90% after chronic neuroleptic treatment. Moreover, the D1 agonist facilitation of yawning was abolished by chronic neuroleptic treatment as was the yawning induced by the intrastriatal administration of LY171555. Yawning inhibition observed after D1 and D2 antagonist treatment was still present after chronic striatal DA sensitization. These results suggest that D1 receptor involvement as well as autoreceptor sensitization play an important role in the abolition of yawning and in certain disease states.

422.6

QUANTITATIVE BEHAVIORAL ANALYSIS OF DA AGONIST/ANTAGONIST INTERACTIONS IN THE RAT. P.K. Randall, R.E. Wilcox, and J.S. Randall. Inst. for Neuroscience, Div. Pharmacol., Univeristy of Texas, Austin, Texas 78713.

Antagonist blockade of an agonist-induced behavioral response is a common technique in behavioral pharmacology. Unfortunately, IC_{50}'s derived from experiments in which increasing antagonist doses are run against a constant agonist dose are dependent not only upon the K_b of the antagonist, but also upon at least 3 other parameters, the K_A and dose of agonist, and the ED_{50} of the particular reponse measured. Determination of an "*in vivo* K_b" for an antagonist using pharmacological null methods should be dependent only upon antagonist characteristics. Since these methods were designed to be used in preparations in which tissue factors remain constant across different agonist and antagonist doses, it is questionable whether any valid information can be obtained with complex response systems such as behavior.

To determine the feasibility of utilizing such techniques in behavioral preparations, we examined the effects of 3 doses of each of 3 DA antagonists on the dose-response curve for apomorphine-induced behavior in Sprague-Dawley rats. Dose-response curves were assessed by non-linear estimation using the null model for competitive antagonists. Fluphenazine, at .1 and .25 mg/kg produced parallel shifts in apomorphine dose response curves suggesting an *in vivo* K_b of .04 to .06 mg/kg, IP. This was the case regardless of which of several behavioral measures were employed (number of animals showing stereotypic behavior, mean maximum stereotypic rating, gnawing). SCH23390 and spiperone affected the dose-response curves in a much more complex manner which was highly dependent upon the particular components of the behavioral response assessed. These data suggest that such techniques may be relatively robust in behavioral experiments when the antagonist employed interacts similarly with all populations of relevant receptor species affected by the agonist. Thus fluphenazine has almost equal affinity for D-1 and D-2 receptors and affects the dose-response curves of an equally non-selective agonist in a simple manner. Antagonists which discriminate between different sub-populations of receptors affected by the agonist (i.e. SCH23390 (D-1) and spiperone (D-2)) not surprisingly exert more complex effects.

422.7

COMPARATIVE EFFECTS OF D_2-DOPAMINERGIC AGONISTS ON COPULATORY BEHAVIOR OF MALE RATS. M.M.FOREMAN, R.D.TITUS* and J.M.SCHAUS*. The Lilly Research Laboratories, Eli Lilly and Company, Lilly Corporate Center, Indianapolis, IN 46285.

The present studies have compared the effects of variety of potent and selective D_2-dopaminergic receptor agonists from different structural groups including an aporphine agonist [R-N-n-propylnoraporphine (PrAPO)], a hydroxy-napthoxazine agonist [(+)PHNO] and "BCD" partial ergolines (quinpirole, quinelorane, LY173452 and LY237917) on male rat copulatory performance. The animals chosen for these studies were 6-12 month old, male Sprague-Dawley rats, which exhibited constant ejaculatory latencies (time interval between intromission and ejaculation, EL) in repeated tests. Subcutaneous injections of solutions containing various concentrations of these dopaminergic agonists were made 30 minutes prior to behavioral testing with a sexually receptive female rat. Although all of these agents were capable of producing significant reductions in EL, there was a 100 fold variance in the minimum effective dose. PHNO, quinpirole and PrAPO required a minimum dose of 2.5 ug/kg to elicit changes in EL, whereas LY173452 required 250 ng/kg and quinelorane and LY237917 required 25 ng/kg. The evaluation of sexual behavior is an extremely sensitive in vivo method for comparing pharmacologic activities of D_2-dopaminergic agonists, which may provide a measure of preclinical efficacy of agents used to treat sexual disorders.

422.8

POTENTIATION BY ANTICHOLINERGICS OF THE INHIBITION BY D1-RECEPTOR ANTAGONISTS OF CONDITIONED AVOIDANCE RESPONDING (CAR) IN RATS. L.C. Iorio, R. Chipkin, M. Cohen-Winston* and V. Coffin*. Schering-Plough Inc., Dept. of Pharmacology, Bloomfield, NJ. 07003.

Many studies have confirmed the first demonstration (Iorio et al, 1984) that the specific D1 antagonist SCH 23390 blocked CAR in rats at relatively low doses. This effect of SCH 23390 was indistinguishable to that seen after specific D2 antagonists, and this raised questions about a possible interaction between these 2 sub-classes of receptors in the CAR.

We have recently found that the overt sedative effects of selective D1- and D2-receptor antagonists in Cebus monkeys can be discriminated by the anticholinergic drug benztropine (Coffin et al, in press). This report demonstrates that CAR blockade in rats by specific D1 and specific D2 receptor antagonists can also be discriminated by anticholinergics. Atropine and scopolamine, at doses which by themselves did not affect CAR, potentiated CAR blockade seen after oral SCH 23390 or SCH 39166 (shift in the D-R curve to the left) but blocked CAR blockade seen after D2 antagonists such as haloperidol (shift of the D-R curves to the right).

These results suggest that the D1- and D2-receptor systems involved in CAR are linked to a cholinergic mechanism in a reciprocal manner.

422.9

ANGIOTENSIN CONVERTING ENZYME INHIBITION AND APOMORPHINE-INDUCED STEREOTYPED BEHAVIOR. A. Sudilovsky, B. Turnbull, L. H. Miller*. Squibb Institute for Medical Research, Princeton, NJ 08540; Boston University, Boston, MA 02215.

Although the angiotensin converting enzyme (ACE) inhibitor captopril does not competitively bind to the dopamine receptor it attenuates apomorphine-induced stereotypy when given one hour prior to apomorphine (Sudilovsky et al, Soc. Neurosci. Abst., 9:1, 132, 1983). The current experiments, using male Sprague-Dawley rats (N=9/group) randomly injected i.p. with ascending doses of captopril (5, 10, 20 and 50 mg/kg) one hour before i.p. administration of apomorphine (2 mg/kg), demonstrated a dose response relationship for the earlier reported effect. Stereotypy was rated in blind fashion using a 0-3 point scoring method (Tarsy and Baldessarini, Neuropharmacol., 13, 927, 1974). Pretreatment of rats with another ACE inhibitor, SQ 29,852 (5 mg/kg), also produced a significant decrease in stereotypy ($p<0.05$), which was of comparable magnitude to that resulting from pretreatment with the antipsychotic dopamine receptor blocker fluphenazine at 1 mg/kg ($p<0.05$). The inhibitory effect of the latter was potentiated by its combined administration with SQ 29,852. In addition, pretreatment with epicaptopril, the virtually inactive diastereoisomer of captopril, did not significantly affect stereotypy.

These data suggest an antagonist interaction between the ACE inhibitors and the dopaminergic system underlying stereotypy and support an involvement of ACE inhibition.

422.10

LOSS OF STRIATAL NEURONS FOLLOWING 8 MONTHS OF FLUPHENAZINE-TREATMENT IN RATS. DV Jeste, JB Lohr, DS Segal, R Kuczinski, PM Groves. San Diego VA Medical Center and Univ. of CA, San Diego, San Diego, CA 92161.

Neuroleptic-induced persistent tardive dyskinesia, is believed to be associated with striatal pathology. Yet, there have been few quantitative neuropathologic studies of sufficiently prolonged neuroleptic treatment. METHODS: We treated 10 male Sprague-Dawley rats with fluphenazine decanoate, 5 mg/kg, (or vehicle) im q 2 weeks for 8 months. Three weeks after last injection, animals were sacrificed. One hemisphere from each animal was used for striatal neuronometry with IBAS-2000 image analysis system, and the other hemisphere for measuring striatal dopamine, HVA and DOPAC concentrations. RESULTS: Fluphenazine-treated animals showed a 46% reduction (p 0.05) in the number of large (15u) neurons (likely to be cholinergic), and a decrease in striatal dopamine (by 28%), HVA (50%) and DOPAC (29%) concentrations. COMMENT: Chronic neuroleptic-induced neurotoxicity is probably associated with a loss or shrinkage of cholinergic neurons and a loss of dopaminergic terminals in striatum.

(SUPPORTED BY VA MERIT REVIEW GRANT TO DVJ)

422.11

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

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

422.12

EFFECTS OF DOPAMINE ANTAGONISTS ON ACQUISITION AND EXPRESSION OF THE AMPHETAMINE CONDITIONED PLACE PREFERENCE Noboru Hiroi and Norman M. White Department of Psychology, McGill University, 1205 Dr. Penfield Ave, Montreal, Quebec, Canada, H3A 1B1

Based on our previous demonstration that dopamine receptor activation is required for expression of the amphetamine conditioned place preference (CPP), we investigated the effect of selective dopamine receptor blockers on both acquisition and expression of the CPP. Using a 3-compartment CPP apparatus, rats were trained with 2 amphetamine (2mg/Kg) pairings in one compartment and 2 saline pairings in another compartment in a counterbalanced manner; they were tested for place preference on the following day. Antagonists were given IP before training trials (acquisition) or before testing (expression). A mixed antagonist (alpha-flupenthixol, 0.2-1.0mg/Kg), a D1 antagonist (SCH23390, 0.02-0.16mg/Kg) and a D2 antagonist (sulpiride, 10.0-120.0mg/Kg) all dose-dependently blocked both acquisition and expression. Higher doses of all drugs were required to block expression than acquisition; the difference was smallest for the D1 antagonist and largest for the D2 antagonist. These findings suggest that activation of dopamine receptors is required for both acquisition and expression of the amphetamine CPP. The fact that higher doses are required to block expression than acquisition suggests that different mechanisms may mediate these two processes.

422.13

EFFECTS OF SELECTED DOPAMINE D1 AND D2 DRUGS ON SCHEDULE-CONTROLLED BEHAVIOR IN RATS BEFORE AND DURING CHRONIC SCH 39166 ADMINISTRATION. V.L. Coffin* and M.B. Latranyi* (SPON:R.E. Chipkin). Schering-Plough Corp., 60 Orange St., Bloomfield, NJ 07003.

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

422.14

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

The D2 agonists quinpirole (Q), pergolide (P) , B-HT920 (BH) and (-)-3-PPP (PPP) produced depression (reduced sniffing, rearing and grooming) in mice using a subjective scoring system. Q-, P- and BH-induced immobility were reversed by the D1 agonists SKF38393 (S) and CY208 243 (CY). Q-induced immobility was only reversed by the active enantiomer of S. PPP-induced immobility was reversed by CY and S, but this was due to an increase in grooming in mice given the D1 agonists, whether or not PPP was also given. There was no reversal of the depression of rearing or sniffing. While CY and S antagonized BH-induced immobility, the reversal was evidenced by partial reversals of the depression of sniffing, rearing and grooming. The S and CY reversal of Q immobility was blocked by the D1 antagonist SCH23390. High doses of SCH23390 and raclopride increased immobility. All the D2 agonists tested, in combination with S, stimulated activity in DA-depleted mice as monitored in automated cages. It is concluded that the D2 agonist-induced depression was due to a deprivation of DA specifically at D1 receptors. Furthermore, the depressant doses of the D2 agonists were able to stimulate the postsynaptic (PS) D2 receptors, which could be demonstrated under appropriate conditions. Thus, D1 agonists reverse D2-induced depression by stimulating the PS D1 receptors thereby taking the place of the endogenous DA. The necessary PS D2 receptor stimulation is provided by the D2 agonist. DA autoreceptor agonists are probably functionally selective because of the PS D1/D2 interaction, rather than displaying an ability to preferentially couple with the autoreceptors. # and (permanent address) Department of Pharmacology, University of Sydney, Australia.

422.15

'PRIMING' PHENOMENON: PHARMACOLOGICAL AND BIOCHEMICAL STUDIES

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'Priming' is a mechanism which strongly potentiates the action of a dopaminergic (DA) agonist in denervated animals. In unilaterally 6-hydroxydopamine lesioned rats in fact, the administration of a DA agonist ('priming') significantly increases the contralateral turning induced by the administration of a D-1 or D-2 agonist. Moreover in primed rats a dose of a D-1 agonist inactive in drug-naive rats become fully effective in eliciting contralateral turning. 'Priming' has a time and dose dependency. It's effectiveness was maximal 3 days after the administration of the primer drug and decreased 10 days later; moreover the response to the D-1 or D-2 agonist was different depending from the dose of the drug used for 'priming'. Turning in response to 'priming' although strictly dependent on the stimulation of supersensitive striatal receptors and on the full expression of the contralateral turning in response to this stimulation, is not influenced by conditioning. The molecular mechanism at the basis of 'priming' was investigated by studying the binding of D-1 receptors and the activity of adenylate cyclase. No changes were found in the number and affinity of D-1 receptors while an increase in the affinity of the adenylate cyclase stimulated by D-1 agonists was observed. Further studies with 2-deoxyglucose autoradiography are in progress in order to assess if 'priming' promotes the activation of specific brain areas.

422.16

D-1 AND D-2 RECEPTORS INTERACTION IN PARKINSONIAN MONKEYS. M.R. Luquin*, T. Herrero*, J. Del Rio*, J.A. Obeso*. (SPON: C. Cuello). Dpt. of Movement Disorders (Neurology) and Pharmacology, University of Navarra, Medical School, Pamplona, Spain.

Severe parkinsonism was induced in 3 monkeys (Macaca Fasciculata) by intravenous injections of MPTP. Six weeks were allowed for recovery and stabilization. The dose-response curve for motor improvement induced by CY 208-243 (D-1 agonist) and (+) PHNO (D-2 agonist) given independently for a minimum of 4 different doses, was studied. Subsequently a sub-threshold dose of CY 208 - 243 was given concomitantly with the (+) PHNO doses previously used. A positive interaction on the duration and intensity of the motor response was found between both drugs as reflected by a shift to the left of the (+) PHNO dose response curve. These results provide further evidence for a functional linkage of D-1 and D-2 receptors mediating motor response in primates with substantia nigra lesion.

422.17

ATTENUATION OF THE DEVELOPMENT OF BEHAVIORAL SENSITIZATION TO SYSTEMIC AMPHETAMINE BY MICROINJECTIONS OF SCH-23390 INTO THE VENTRAL TEGMENTAL AREA AND SUBSTANTIA NIGRA PARS RETICULATA. J. Stewart and P. Vezina. Center for Studies in Behavioral Neurobiology, Department of Psychology, Concordia University, Montreal, Canada, H3G1M8.

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

422.18

THE BEHAVIORAL AND NEUROCHEMICAL EFFECTS OF S-ADENOSYL-METHIONINE (SAM) IN RODENTS. J. M. Beaton, L. C. Tolbert, F. Antun* and A. M. Freeman*. Neuropsychiat. Res. Prog., Dept. Psychiat., Univ. of AL. at B'ham, B'ham., AL. 35294.

There is clinical interest in the use of SAM as an antidepressant and in the treatment of hyperactivity. We were interested in examining the effects of SAM on various mouse and rat behaviors. Several doses of SAM were tested after acute or chronic sub-cutaneous administration. Acute doses of 100,200,400 or 800 mg/kg SAM had no significant effects on analgesia, passive avoidance or open-field activity in mice. However, acute administration of SAM plus 4 mg/kg amphetamine did slightly increase the open field activity, over amphetamine alone. Chronic administration of 100,200,400 or 800 mg/kg SAM did significantly increase open-field activity in mice, after 4, 7 or 10 days of administration. The effects of 4 mg/kg amphetamine after 4 days of SAM were significantly increased over the effects of amphetamine alone. In rats with chemical lesions in the nigro-striatal dopamine pathway, treatment with 200 mg/kg SAM for 10 days, compared to saline, potentiated amphetamine (2 mg/kg) induced turning behavior. After testing, the rats were sacrificed and the caudates excised from the lesioned and intact hemispheres of both groups. The caudates were assayed for D_2 agonist binding. There was a decrease in B_{max} found in the lesioned caudates for both groups, as would be expected. An increase in B_{max} was found in the SAM treated animals in both the lesioned and non-lesioned caudates when compared to the appropriate saline control. These data indicate that SAM does modulate the dopamine system by increasing receptor density. (We acknowledge Bioresearch, S.P.A., Milan, Italy for providing the SAM).

422.19

EFFECTS OF AMPHETAMINE OR PARA-HYDROXY-AMPHETAMINE ON THE BEHAVIOR AND DOPAMINE LEVELS IN THE NEOSTRIATUM OF THE RAT

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We examined the effects of pretreatment with amphetamine (AMPH) or para-hydroxy-amphetamine (P-OH-AMPH) on the ability of AMPH to release dopamine (DA) in the neostriatum and to stimulate locomotor activity or stereotyped behavior. Rats were injected with either AMPH (2 mg/kg) once daily for three days, a single dose of P-OH-AMPH (10 mg/kg) or saline (1 ml/kg) as pretreatment. On the following day DA release and behavioral responses to a challenge dose of AMPH (2 mg/kg) were examined. In vivo microdialysis samples were collected every 10 minutes and analysed for DA, DOPAC and HVA using HPLC-EC. Locomotor activity and stereotyped behavior were recorded every 10 minutes in an open field chamber equipped with photo cells. P-OH-AMPH pretreatment enhanced the release of DA compared to saline pretreatment. Both AMPH and P-OH-AMPH pretreatment decreased locomotor activity and increased stereotyped behavior compared to saline pretreatment. Pretreatment with P-OH-AMPH, a metabolite of AMPH, can enhance AMPH enduced DA release as well as produce typical behavioral changes seen with AMPH pretreatment. These results indicate that P-OH-AMPH is responsible for part of the dopaminergic and behavioral changes seen with AMPH pretreatment. USPHS Grant DA03020.

423.1

CHRONIC AND ACUTE PROGLUMIDE: ITS EFFECTS ON AMPHETAMINE INDUCED BEHAVIORS. W.E.MONTANA, M.CULLEN AND K.E.ASIN. Neurosci.Res.Div., Pharmaceutical Discovery,Dept.47H Abbott Labs., Abbott Park, Il 60064.

The CCK antagonist proglumide (PROG), given chronically, has been reported to increase the number of [3H]spiperone binding sites in the nucleus accumbens of the rat, which has also been seen after chronic administration of some neuroleptics. Given acutely, PROG has been shown to enhance haloperidol's (HAL) inhibition of apomorphine induced stereotypy. This experiment investigated the effects of chronic and acute PROG administration on dopamine mediated (amphetamine-induced) locomotion and rotation.

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

423.2

EFFECT OF A-68552, A CENTRALLY ACTIVE CCK AGONIST, ON CONDITIONED AVOIDANCE RESPONDING IN MICE, RATS AND MONKEYS. P. Curzon, M.D. Tufano*, A.M. Nadzan* and D.R. Britton. Neuroscience Res. Div., Pharmaceutical Discovery, Dept. 47H, Abbott laboratories, Abbott Park, ILL. 60064

A-68552 is a potent, centrally active Type A CCK receptor agonist. In mice (icv) it is 300 times as active as CCK-8-S in suppressing locomotion. CCK-8-S has been shown to reduce avoidance responding in the rat (Cohen et al.1982) at doses above 320 ug/kg i.p. We have tested A-68552, CCK-8-S as well as typical and atypical antipsychotics for disruption of avoidance responding in mice, rats and cynamologus monkeys.

In mice A-68552 was tested at up to 1000 nmol/kg i.p. with minimal disruption of the avoidance response; haloperidol was effective at 0.1 and 0.2 mg/kg i.p. In the rat A-68552 was tested on both acquisition and performance of the response both sc and icv with no significant effects. A comparison was made to both typical and atypical antipsychotics in the same paradigms. A-68552 was tested i.m. in the monkey conditioned avoidance at doses up to 200 nmol/Kg with no decrease in responding.

In summary, a potent CCK agonist fails to show antipsychotic-like activity in these preclinical tests.

423.3

EFFECTS OF THE CCK AGONIST A68552 ON AMPHETAMINE CONDITIONED ACTIVITY. K.E.Asin, L.Bednarz*, M.Tufano* & A.Nadzan*, Neuroscience Research Division., Pharmaceutical Discovery, D-47H Bldg AP10, Abbott Labs, Abbott Park, IL 60064.

A68552 is a CCK-7 analogue which we have shown to have nanomolar affinity for pancreatic CCK receptors but is more metabolically stable than is CCK. We used A68552 to investigate possible CCK/dopamine (DA) interactions in the acquisition or expression of environment specific conditioned locomotor activity (CLA) produced by d-amphetamine (Am). During 5 days of conditioning trials, male rats were injected with Am (0 or 2.5mg/kg,ip) and A68552 (0 or 100 ug/kg,ip) before being placed into locomotor activity boxes (AB) for one hour; controls received identical treatments after being removed from the boxes. On the test day, all animals were injected with either vehicle or A68552 1 min before being placed into the ABs. Compared to the appropriate control groups, Am injections prior to AB placements during conditioning produced significant CLA and rearing; these effects were not reduced by coadministration of A68552 during conditioning. However, administration of A68552 prior to testing significantly reduced both Am conditioned activity and rearing. In other animals, neither 10, 100 nor 1000 ug/kg A68552 suppressed the unconditioned locomotor response to Am. Our CLA results are directly opposite to those reported by Beninger and Hahn (1983) using the DA antagonist pimozide, and may suggest the involvement of CCK mechanisms in the expression of learned behaviors.

423.4

CCK FACILITATES ACQUISITION OF A DISCRIMINATED AVOIDANCE RESPONSE. J.Rodenhiser*, D.Grider*, M. Shuck*, and E.Quinton (SPON: R.Smith). Dept. of Psychology, University of Louisville, Louisville, Ky.40292.

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

423.5

BEHAVIOURAL EVIDENCE THAT CHOLECYSTOKININ IS A FUNCTIONAL DOPAMINE ANTAGONIST IN THE NUCLEUS ACCUMBENS. C.Y.Yim and G.J.Mogenson, Dept. of Physiology, Univ. of Western Ont., London, Ontario, Canada, N6A 5C1.

Previous electrophysiological experiments showed that sulphated cholecystokinin (CCK) blocks the neuromodulatory actions of dopamine (DA) in accumbens (NAcc) neurones (Yim et al, Soc. Neuro. Abst. 14(1988)114). The present study investigates whether or not those observations have functional behavioural significance. Male Wistar rats were implanted with injection cannulae into the ventral tegmental area (VTA) and the NAcc. DA-mediated hyperlocomotor activity was produced by injection of picrotoxin (PIC), a GABA antagonist, into the VTA (Brain Res. 161(1979)311). The effects of simultaneous injection of CCK or its antagonist, proglumide (PRG), into the NAcc was investigated. At a dose of 25 ng, PIC produced 31% increase in locomotor activity (n=42) and at 50 ng, a 272% increase. PRG (30 ug) injection into the NAcc did not produce any significant change in activity by itself but enhanced the PIC (25 ng dose, n=25) induced activity by 17%. CCK (40 ng) injection into the NAcc produced an 18% increase in activity by itself but significantly attenuated (-36%) the PIC (50 ng, n=17) induced locomotor activity. These results show that CCK antagonizes the hyperlocomotor effects of endogenous DA, and suggest that endogenous CCK may be a functional antagonist of DA in the NAcc, a hypothesis which is consistent with conclusions from electrophysiological expts.

423.6

CCK MICROINJECTED INTO THE NUCLEUS ACCUMBENS ATTENUATES AMPHETAMINE-INDUCED FEEDING. T. L. Sills and F. J. Vaccarino. Department of Psychology, University of Toronto, Toronto, Ont., M5S 1A1.

Systemically administered d-amphetamine (AMP), in low doses, enhances intake of foods high in sucrose content. Further evidence indicates that this effect is dopaminergic and that the nucleus accumbens (Acb) may be an important site of action. Of interest here is the fact that CCK terminals exist within the Acb and that neuroleptic-like behavioral effects have been reported following intra-Acb CCK microinjections. The present study tested the effects of intra-Acb CCK on AMP-induced feeding.

Male Wistar rats were presented with both powdered chow and sugar in their home cages and baseline intake was measured for one hour each day for six days. On subsequent days, rats were taken from their home cages and injected with either saline or AMP (0.125 mg/kg) followed by CCK injections into the rostral Acb in doses of 0, 1 and 2 ng. Rats were then returned to their home cages and offered both chow and sugar; intake was recorded for one hour.

Consistent with previous results, the administration of AMP resulted in an increase in the intake of sugar relative to vehicle injection. Both doses of CCK attenuated the increase in sugar intake to the extent that intake returned to vehicle baseline levels. These results support the notion that the Acb is an important site of action for AMP-induced feeding and further indicate that Acb CCK may play a modulatory role in this effect.

This research was supported by a NSERC grant to FJV.

423.7

CHOLECYSTOKININ REDUCES BODY TEMPERATURE AND FOOD INTAKE IN VEHICLE BUT NOT CAPSAICIN PRETREATED RATS. E.H. South, R.C. Ritter and P.D. Huff*. Dept. Vet. Sci., Univ. of Idaho, Moscow, ID 83843 & Dept. of VCAPP, Washington State Univ., Pullman, WA 99164-6520.

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

		SALip		CCK4ugip	
TRT	MIN	RT °C.	FOOD g.	RT °C.	FOOD g.
CAP	20	0.8±0.2	2.4±0.3	0.6±0.1	3.0±0.4
VEH	20	0.3±0.3	4.0±0.7	-0.3±0.3**	1.8±0.6#

(all means ± SE; ** p<.02, RT significantly decreased;
# p<.05, food intake significantly reduced;
MIN, time post CCK or saline injection)

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

423.8

CCK-8 EFFECTS ON SLEEP AND FEEDING. A.Posadas-Andrews[1,3]*, O. Prospéro-García[2] and J.A. Rojas-Ramírez[1]* [1]Depto. Farmacología, Medicina and [2]Depto. de Neurociencias, Instituto de Fisiología Celular. [3]ENEP-Iztacala, UNAM, Apdo. Postal 70-600, México 04510, D.F. México.
(SPON: F. Velasco)

It has been reported that central or peripheral administration of cholecystokinin (CCK-8) induces sleep and inhibits food-ingestion in the rat. To study the relationship between these two behaviors, we treated normal and food-deprived rats with either saline or CCK-8 (10 ug/kg i.p.). Sleep recordings (3 h) started immediately after injection; at this time, rats had free access to food and water. CCK-8 decreased wakefulness and increased slow wave sleep (SWS) in all animals whereas REM sleep increased in sated-CCK-8 rats and did not change in the food-deprivation group. CCK-8 decreased latency to slow wave sleep, without change in REM sleep latency in sated rats, while food-deprivation-saline, reduced it, and increased latency to SWS. A combined effect was observed in the CCK-food-deprived group, CCK-8 did not modify the amount of food-ingested. We suggest that CCK-8 effect on food-ingestion may be secondary to the CCK-8-induced sleep.

NEUROPEPTIDES AND BEHAVIOR: CRF

424.1

EVIDENCE FOR INVOLVEMENT OF SEROTONIN IN BEHAVIORAL EFFECTS OF CRF. D.R. Britton, E. Indyk* and A. Lazosky*, Dept. Pharm. and Molecular Biol., Univ. Health Sciences/ Chicago Medical School, North Chicago, IL 60045.

Intraventricular (icv) corticotropin-releasing factor (CRF) elicits a variety of responses associated with "stressful" stimuli. These include suppression of eating and increased grooming. The locomotor response is enhanced in the home cage (HC) but suppressed in a novel modifiefd open field (MOF). We have examined the effects of the 5-HT depleting agent, 5,7-dihydroxytryptamine (5,7-DHT) on the subsequent response to CRF.

Male rats were given icv cannulas and injected icv with either saline or 5,7-DHT (200ug in 10ul). At least 10 days they were injected icv with saline or rCRF in doses of 60 or 120 pmol and observed for the following 60 min. in the HC or for 15 min in the MOF. In HC, 5,7-DHT alone increased locomotion to a level seen with CRF administration. There was no apparent additive effect of the two. In the MOF, animals previously give icv saline showed behavioral suppression in response to icv CRF. Animals which had been treated with 5,7-DHT were less active than the saline controls, but responded to icv CRF with increased activity. These data suggest that 5-HT plays a role in the expression of CRF-induced behavior.

424.2

EFFECTS OF CHRONIC CRF ADMINISTRATION: BEHAVIORAL, HORMONAL AND RECEPTOR CHANGES. L. Conti*, D. Costello*, R.Loftus*, L. Martin*, M.White* and M. E. Abreu*(SPON: W. Pickworth). Nova Pharmaceutical Corp., Baltimore, MD 21224.

Corticotropin releasing factor (CRF) is a physiological initiator of the stress response which includes the release of ACTH and the subsequent release of corticosterone in animals. Behaviorally, acute administration of CRF (icv) is anxiogenic in a number of models of rodent behavior including a footshock-induced freezing behavior model. We studied the effects of chronic administration of CRF (icv) on this response to footshock in rats. The ACTH and corticosterone responses to CRF were also examined in these chronically treated animals. Finally, CRF receptor binding in cortex of the chronically-treated animals was determined.

Male Sprague-Dawley rats received an icv injection of either ovine CRF (1.0ug in 5ul) or vehicle (5ul BSA 0.1% in saline) once a day for 10 days. On day 11, the two groups were divided so that half of the chronic CRF- and half of the chronic BSA-treated animals were given an acute challenge of CRF (1.0ug) 30 min prior to behavioral testing. The other half of each chronic treatment group received vehicle. For behavioral testing, animals were individually placed in a standard conditioning chamber and observed for a 7.5 min session. One and 1.5 min into this session each rat received a footshock (.5mA, .5sec) through the grid floor of the chamber. Five min after behavioral testing, rats were decapitated , trunk blood collected and brains were removed; cannula placement was verified and cortex and pituitary were taken.

Rats which were challenged with CRF spent more time freezing (immobile) after footshock than vehicle-treated animals. The response was greatest in rats which had been chronically treated with CRF.

424.3

CRF ACTS VIA A THIRD VENTRICLE SITE TO REDUCE EXPLORATORY BEHAVIOR IN RATS.
C. W. Berridge, F. Spadaro* and A. J. Dunn. Dept. Pharmacology, Louisiana State University Medical Center, Shreveport, LA 71130.

Corticotropin-releasing factor (CRF) may play a major role in coordinating the response of an organism to stress. In addition to its ability to activate the hypothalamic-pituitary-adrenal (HPA) axis, ICV CRF activates the sympathetic nervous system and cerebral catecholaminergic systems. ICV administration of CRF elicits neurochemical, physiological and behavioral responses that resemble those observed in stress. It increases locomotor activity and grooming, and decreases sexual behavior and feeding. In the multicompartment testing chamber (MCC), ICV CRF decreases the mean contact time with novel stimuli. This behavioral change resembles that observed following restraint. The CRF antagonist, alpha-helical CRF_{9-41}, reverses this effect of restraint. To determine the site of action of CRF, we injected 20 ng into the lateral or the fourth ventricle of rats using plugs of cold cream to block its access to various parts of the ventricular system. Blockade of the cerebral aqueduct prevented the behavioral response to fourth ventricle CRF, but not to lateral ventricle injections. Moreover, the response to lateral ventricle infusion of CRF was prevented when cold cream blocked its access to the anteroventral quadrant of the third ventricle (AV3V). These results indicate that the site of action of intracerebrally administered CRF on exploratory behavior in the MCC is not in the fourth ventricle, and suggest that the AV3V may be involved. This result is compatible with the sites of action of CRF in stimulating locomotor activity, and with the sites of action of other ICV-injected peptides.

Supported by a grant from NINCDS (NS27283)

424.4

SELECTIVE PROCONFLICT EFFECT OF CORTICOTROPIN-RELEASING FACTOR (CRF). E.F. Aulisi, R.G. Wehby*, J.L. Katz* and R.J. Valentino. Department of Pharmacology, George Washington University Medical Center, Washington, D.C. 20037, and NIDA Addiction Research Center, Baltimore, Maryland 21224.

CRF has been shown to suppress both punished and nonpunished food reinforced responding at similar doses. To determine whether CRF has selective proconflict effects, rats were trained to lever press for food on a multiple fixed ratio schedule in which different levels of shock were used to suppress responding. In one component of the schedule (nonpunishment), 30 responses resulted in food reinforcement (FR30). In the 2nd component (punishment), food was presented after 30 responses, however, the first lever press of the FR30 produced either a level of shock capable of suppressing responding (HS, 0.8 - 1.0 mA), a subthreshold level of shock not capable of suppressing responding (LS, 0.05-0.15 mA), or no shock (NS). These levels of shock were in effect during different sessions. The pattern of responding was such that during LS sessions, rates of both punished and nonpunished responding were similar, while during HS sessions, rates of punished responding were dramatically reduced compared to nonpunished responding. CRF (0.1 - 5.6 μg, i.c.v.) was administered 15 min prior to sessions in which LS was in effect. CRF caused a dose-dependent decrease in both nonpunished and punished responding. However, the dose-response curve for CRF effects on punished responding was shifted to the left of that for nonpunished responding. These results suggest that CRF has selective proconflict effects in rats trained on this schedule. Supported by PHS Grants MH 40008 and MH 42796.

424.5

CORTICOTROPIN RELEASING FACTOR ENHANCES THE ACOUSTIC STARTLE REFLEX: INVOLVEMENT OF THE AMYGDALA AND THE SPINAL CORD. K.C. Liang, M.J.D. Miserendino*, K.R. Melia* & M. Davis. Dept. of Psychiatry., Yale Univ., Sch. of Med., 34 Park St, New Haven, CT 06508

Corticotropin releasing factor (CRF) is implicated in stress- or fear-related behaviors. Intracerebroventricular (icv) injection of CRF enhances the startle reflex (Swerdlow et al., Psychopharmacol., 1986, 88, 147-152), but the site of action of this effect has not been localized. Previous findings indicate that the amygdala is involved in fear- or shock-potentiation of startle, and that the spinal cord mediates the startle facilitatory effects of certain drugs. Because CRF immunoreactivity and receptors are found in these structures, we investigated effects of intrathecal and intracisternal injections of CRF on startle as well as the effect of icv CRF on startle in amygdala-lesioned rats.

Startle was elicited by 95db, 50ms noise bursts (ISI=30s, background noise 55db). Fifteen min after the first stimulus, artificial CSF or CRF was remotely infused into the ventricle or lumbar spinal cord. CRF (0.5 or 1.0 µg) injected icv or intracisternally produced a profound, dose-dependent enhancement of startle, while intrathecal injection of 1.0 µg CRF produced a small increase. Lesions of the amygdala significantly attenuated the effect of icv CRF (1.0 µg). The magnitude of enhancement produced by icv CRF in the amygdala-lesioned rats was comparable to that produced by intrathecal CRF in intact rats. These findings suggest that part of the icv CRF enhancement of startle may result from a direct action on spinal neurons and that the amygdala may be the primary site of CRF action or a critical part of the neural circuitry mediating icv CRF augmentation of startle. Experiments are in progress to investigate the effects of local CRF infusions into brain regions with CRF receptors.

424.6

BEHAVIORAL DISRUPTION WITH CENTRAL ADMINISTRATION OF CORTICOTROPIN RELEASING FACTOR (CRF) IN PIGEONS IS ATTENUATED WITH REPEATED DOSING.
S. T. Ahlers, L. Zhang*, & J. E. Barrett. (SPON: D. C. Riccio) Environmental Medicine Department, Naval Medical Research Institute and Department of Psychiatry, Uniformed Services University of the Health Sciences, Bethesda, MD 20814.

Intracerebroventricular (ICV) administration of o-CRF in pigeons produces dose dependent suppression of schedule controlled responding which, with repeated dosings, diminishes over 3-4 days. This tolerance effect is no longer observed after a 10 day hiatus in which CRF is not administered (Barrett et al.; JPET, in press). In the present experiment 8 pigeons were injected with 30µ/Kg o-CRF ICV either 30 min before (n=4), or just after (n=4), performing on a fixed ratio (FR) 30 schedule for food presentation for 3 consecutive days; on the 4th day all subjects received CRF before the session. A 2 week period in which CRF was not administered separated counterbalanced replication of these conditions. Over the 4 day period, pigeons that received CRF before the session displayed a gradual attenuation of the initial suppressive effects of CRF; responding on day 1 was significantly suppressed whereas responding on the forth day was not significantly different from baseline performance levels. Pigeons that received CRF after the sessions on days 1-3 and then CRF before the session on day 4 showed no suppression of responding on day 4. These data suggest generalized tolerance to the suppressive effects of CRF independent of behavioral variables. Supp. by PHS Grant DA-02873 and USARMDC Contract 86PP6814.

424.7

DOES CRF CONTRIBUTE TO THE COGNITIVE CHANGES SEEN IN DEPRESSION? T.L. Wall, R.I. Chaplin*, C.L. Ehlers. Department of Neuropharm., Research Institute of the Scripps Clinic, La Jolla, CA 92037

Recent studies have suggested that major affective episodes may be accompanied by cognitive impairment in some patients. Electrophysiological studies utilizing event related potentials (ERPs) have also reported that some late component amplitudes (P2, P3) which are associated with cognitive events are also affected during depressions. One neurohormonal factor which is known to be dysregulated in depression, and which may modify cognition is corticotropin releasing factor (CRF). In the present study we sought to evaluate the effects of CRF on ERPs in the rat in order to see whether CRF could produce changes in ERP morphology similar to those seen in human depression, using an auditory oddball plus novel ERP paradigm. Eight male Wistar rats were chronically implanted with electrodes stereotaxically placed in the frontal cortex and dorsal hippocampus (DHPC). Rats were administered saline and CRF intracerebroventricularly (ICV) in doses of 0.01, 0.1, and 1.0 µg per rat. The lowest dose of CRF (0.01 µg) produced significant reductions in the amplitude of the P2 component in cortex. Larger doses of CRF produced increases or no change in the P2 amplitude in cortex and significant dose dependent decreases in the P2 and P3 components in DHPC. These data are consistent with the idea that increases in CRF activity may contribute to the cognitive changes seen in depression. (Supported my NIAAA 00098, 06059, and the MacArthur Fnd.)

424.8

REVERSAL OF A NONMONOTONIC RELATIONSHIP BETWEEN FOOTSHOCK OR CRF AND THE ACOUSTIC STARTLE REFLEX BY BRIEF EXPOSURE TO A MILD STRESSOR. K.R. Melia*, K.C. Liang, M.J.D. Miserendino*, M. Davis. (SPON: T. Baker). Dept. of Psychiatry, Yale Univ. Sch. of Med., 34 Park St., New Haven, CT 06508.

Acute and chronic stressors potentiate the acoustic startle response in a number of species. The present study evaluated the relationship between the intensity of a stressor and subsequent modulation of startle amplitude using two different stressors: footshock and intraventricular (ICV) injections of the stress related peptide, corticotropin releasing factor (CRF).

Three groups of rats received ten 500 ms shocks (1/5 min) per day on 3 consecutive days. Shock intensity was 0.2, 0.6 or 1.4 mA depending on group. Startle amplitude was tested 2 days later in the chamber where shock had been presented. Other groups received ICV infusion of 0.1, 1.0 or 10.0 µg CRF and startle amplitude was measured over the next hour. A nonmonotonic relationship was found between both footshock intensity and CRF concentration and startle amplitude. Specifically, experience with 0.6 mA footshock or infusion of 1.0 µg CRF significantly facilitated startle amplitude whereas both higher and lower levels of shock or concentrations of CRF did not. Most striking is that a mild stressor (sub-cutaneous saline injection or mild, brief footshock) presented 15 min prior to testing produced a marked enhancement of startle amplitude only in animals exposed to 1.4 mA shock or 10.0 µg CRF resulting in a monotonic relationship between startle and the intensity of prior stress. Thus, the apparent inability of high levels of stress to potentiate startle is due to the active inhibition of an underlying facilitatory effect which can be released by mild stress.

NEUROPEPTIDES AND BEHAVIOR: OXYTOCIN AND VASOPRESSIN

425.1

EFFECTS OF HEXAPEPTIDE METABOLITES OF VASOPRESSIN (AVP) AND OXYTOCIN (OT) ON SPATIAL MAZE PERFORMANCE, FEAR CONDITIONING, AND BRAIN CATECHOLAMINE LEVELS IN THE RAT. J.D. Stoehr*, C.P. Cramer and W.G. North*, Department of Physiology, Dartmouth Medical School and Department of Psychology, Dartmouth College, Hanover, NH 03756.

It has been previously shown that AVP enhances while OT attenuates certain behavioral processes such as learning and memory (deWied, 1987). In the present studies we investigated the influence of C-terminal fragments of these peptides on brain neurotransmitter levels and their possible effects in other behavioral paradigms.

Intracerebroventricular administration of 0.5 pg AVP (4-9) hexapeptide had no apparent effect on the acquisition of a food reward on the eight-arm radial maze. There was no significant difference between AVP (4-9) and saline-treated animals in the number of spatial memory errors that occurred within each day's first eight arm choices; subsequent central treatment with 1 pg OT (4-9) had no significant effect on the number of errors after a previous error-free criteria had been reached.

However, both post-training and pre-retention peripheral injections of 0.3 µg of these hexapeptides significantly altered observable defensive freezing behavior of rats placed in conditioning chambers that 24 hours previously were paired with footshock. OT (4-9) treated animals froze significantly less, 12-16% of time observed, than controls, 27-29% and AVP (4-9) treated animals, 38%. Because AVP (4-9) alone, without shock, did not elicit freezing, and both peptides were effective when administered post-training, it is possible that they are affecting memory processes and not emotional or autonomic responses.

Further studies revealed that subcutaneous injections of 0.3 µg AVP hexapeptide significantly alters catecholamine turnover in certain limbic structures of the brain as determined by HPLC analysis. For instance, AVP (4-9) treatment increased the content of norepinephrine in the central amygdala and substantia nigra, dopamine and 5-HT in the hippocampus, and lowered norepinephrine in the locus coeruleus.

These studies indicate that the hexapeptide metabolites of oxytocin and vasopressin selectively modulate certain behaviors and possibly exert these effects through a mechanism that includes altering brain amine levels.

425.2

CHRONIC CENTRAL ADMINISTRATION OF VASOPRESSIN ANTAGONIST HASTENS EXTINCTION OF CONDITIONED TASTE AVERSION IN RATS. M. D. Brot, I. L. Bernstein and D. M. Dorsa. GRECC, VA Med. Ctr., and Depts. of Psychology, Pharmacology, and Medicine, Univ. of Washington, Seattle, WA 98108

The neuropeptide vasopressin (AVP) has been implicated as a memory enhancing hormone in studies of passive and active avoidance. Previous studies administering AVP in a single dose intraventricularly have shown facilitated learning. Conversely, the centrally active AVP receptor antagonist $d(CH_2)_5Tyr(Me)AVP$ impairs memory processes when injected acutely. In this study, we have examined the effect of chronic central treatment with AVP or its antagonist on retention using a conditioned taste aversion (CTA) paradigm.

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

425.3

COORDINATION OF HAMSTER LORDOSIS AND FLANK MARKING: ROLE OF ARGININE VASOPRESSIN (AVP) WITHIN THE MEDIAL PREOPTIC-ANTERIOR HYPOTHALAMUS (MPOA-AH). S. Rawls* & H.E. Albers (SPON: R. Sridaran). Lab. Neuroendocrinol. & Behav., Depts. Biol. & Psychol., Georgia State Univ., Atlanta, GA 30303.

The present study examined whether AVP within the MPOA-AH is involved in controlling the inverse relationship between lordosis and flank marking normally observed during the estrous cycle. AVP, but not saline microinjected into the MPOA-AH of ovariectomized (OVX) hamsters stimulated high levels of flank marking during tests with a sexually experienced male, or when tested alone. In contrast, AVP microinjected into the MPOA-AH of OVX hamsters given estradiol benzoate (EB) and progesterone (P) did not stimulate flank marking or inhibit lordosis during tests with a male. However these same females exhibited high levels of flank marking in response to AVP when tested alone. A second experiment demonstrated that P was not required for inhibition of AVP induced flank marking in OVX females given EB and tested with males. The present study provides no evidence that AVP acts within the MPOA-AH to inhibit lordosis, but demonstrates that ovarian hormones and male social contact block the induction of flank marking by AVP microinjected into the MPOA-AH. These data suggest that one component in the neural coordination of lordosis and flank marking is inhibition of the response of the MPOA-AH to AVP. (Supported by NSF BNS-8711373).

425.4

LOCALIZATION OF ARGININE VASOPRESSIN (AVP) INDUCED FLANK MARKING IN THE FEMALE HAMSTER HYPOTHALAMUS. W.T. Solomon*, S.Y. Liou*, & H.E. Albers (SPON: K. Wallen). Lab. Neuroendocrinol. & Behav., Depts. Biol. & Psychol., Georgia State Univ., Atlanta, GA 30303.

Microinjection of AVP into the hamster hypothalmus induces intense bouts of flank marking (FM) behavior. Mapping studies of AVP responsive sites in males have shown that AVP acts within the medial preoptic-anterior hypothalamic continuum (MPOA-AH). Since the MPOA-AH is sexually dimorphic the present study examined the sites where AVP induces flank marking in females. Female hamsters (n=21) were stereotaxically implanted with a guide cannula aimed at sites ranging from 2.2mm anterior to 0.1mm posterior to bregma. After surgical recovery, each animal was tested for AVP (9uM in a 20nl saline) induced FM during a 10 min test. Following the experiment the brains of all animals were histologically prepared to determine the site of AVP injection under light microscopy. The amount of flank marking observed ranged from 0-76/10 min. Forty-three% of the sites injected produced no response (0-5FM); 14% produced a partial response (6-19FM); and 43% produced a full response (>20FM). Sites producing full and partial FM responses were found throughout the MPOA-AH continuum. In summary, these data confirm that AVP stimulates flank marking in female hamsters, and suggests that the site of AVP action is similar to that in male hamsters. (Supported by NSF BNS-8711373).

425.5

NEUROPEPTIDES, PASSIVE AVOIDANCE BEHAVIOR AND THE IMMUNE SYSTEM. G. CROISET*, C.J. HEIJNEN* AND D. DE WIED* (SPON: A. Croeze). Rudolf Magnus Institute for Pharmacology, Medical Faculty, University of Utrecht, the Netherlands.

The impact of emotional stimuli on the reactivity of the immune system was studied in rats. To that end a one-trial learning passive avoidance test was chosen. As an in vivo immunological parameter a primary antibody response against Sheep Red Blood Cells (SRBC)(number of antibody secreting cells in the spleen (PFC)) was used.

Exposure of rats to the passive avoidance apparatus resulted in an increase in primary antibody response (apparatus control group). A decrease in primary antibody response was demonstrated in rats that showed passive avoidance behavior; an inverse relationship between avoidance latencies and the magnitude of a primary antibody response was observed.

Neuropeptides affect avoidance behavior. The effect of altered avoidance behavior, as a consequence of neuropeptide administration, on the immune response was studied. DGAVP facilitated avoidance behavior (avoidance latency control 62 sec. vs DGAVP treated rats 300 sec.; in DGAVP treated rats a decrease in number of PFC's was observed (control 930.10^6 PFC vs DGAVP treated rats 403.10^6 PFC). ACTH(4-10) also facilitated avoidance behavior (avoidance latency control 49 seconds vs ACTH(4-10) treated rats 300 sec.) in these rats however the inverse relationship between avoidance latency and number of PFC was disturbed (control 1300.10^6 PFC vs ACTH(4-10) treated rats 1900.10^6 PFC). CRF, intracerebroventricular administered, attenuated passive avoidance behavior (control 300 sec. vs CRF treated rats 44 sec.); in CRF treated rats an increase in number of PFC was observed (control 125.10^6 PFC vs CRF treated rats 425.10^6 PFC).

The involvement of the autonomic system in modulating the immune response will be discussed.

425.6

VASOPRESSIN-SENSITIVE NEURONS IN THE LATERAL SEPTUM AND BED NUCLEUS OF THE STRIA TERMINALIS ARE INVOLVED IN THE CONTROL OF FLANK MARKING BEHAVIOR IN GOLDEN HAMSTERS. R.W. Irvin*, P. Szot, D. Dorsa, C.F. Ferris. Physiology Dept., Univ. Mass. Med. Ctr., Worcester, MA 01655

It is known that the microinjection of arginine vasopressin (AVP) into the lateral septum, an area shown to have a high density of V1-receptors, has a pronounced effect on memory consolidation and temperature regulation in rats. The present study showed that microinjections of AVP into the lateral septum (LS), as well as the bed nucleus of the stria terminalis (BNST) were able to stimulate flank marking, a form of olfactory communication that functions to maintain dominant/subordinate relationships in hamsters. Microinjections of AVP (1ng/100nl), but not oxytocin or angiotensin, into the LS and BNST significantly elevated flank marking behavior, while microinjections outside these immediate areas were ineffective. In vitro receptor autoradiography showed a clear relationship between the effective sites of microinjection and the density of V1-receptors. It would appear that vasopressin-sensitive neurons in the LS and BNST are necessary for the expression of naturally-elicited flank marking since the microinjection of a V1-receptor antagonist into these sites was able to temporarily block flank marking triggered by the odors of conspecifics. Work was supported by grant #NS23657.

425.7

CONCENTRATIONS OF ARGININE VASOTOCIN (AVT) ARE SEXUALLY DIMORPHIC IN AMPHIBIAN BRAIN. S.K. Boyd* and F.L. Moore (SPON: R. Kingsley). Univ. of Notre Dame, Notre Dame, IN 46556 and Oregon State Univ., Corvallis, OR 97331.

Since the peptide AVT is a potent activator of reproductive behaviors in amphibians, we used Palkovits' microdissection technique combined with RIA (Zoeller & Moore, Neuroendo. 42:120, 1986), to determine concentrations of this peptide in the brain of two species of amphibians.

Brains were collected from sexually mature bullfrogs (Rana catesbeiana; n=10/sex) and rough-skinned newts (Taricha granulosa; n=12/sex). In bullfrogs, AVT concentrations were significantly greater in males, compared to females, in the amygdala pars lateralis, optic tectum, and tegmentum. Concentrations of AVT in the bullfrog nucleus of the solitary tract were significantly greater in females, however. In the newt, AVT concentrations were significantly greater in males in the tectum and interpeduncular nucleus.

In conclusion, concentrations of AVT are sexually dimorphic in some regions of the bullfrog and newt brain. AVT in these regions may play a role in the control of sexually dimorphic behaviors in these species. (Supported by NIH HD24653 and HD13508.)

425.8

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

We have previously shown that progesterone (P) increases the area of oxytoxin (OT) receptor binding within the ventromedial hypothalamus in estrogen primed female rats by using the method of quantitative receptor autoradiography. Estradiol (E) induces OT binding within the ventromedial nuclei of the hypothalamus and surrounding area. P in turn causes the induced OT receptors to spread over OT fibers. By using the OT anatagonist [$d(CH_2)^5$, $[Tyr(Me)]^2$, Thr^4,$Tyr\text{-}NH_2^9$]-[I^{125}]OVT as a ligand, we recently found that the P-dependent spread of OT receptors is not limited to the ventromedial hypothalamus but that P treatment also causes a 80% increase in the area covered by OT receptors within the central amygdala. These results show that the P-dependent spread of OT receptors corresponds to a general mechanism of P action which can be observed within different hormone sensitive brain regions. The spread of OVT binding in both brain regions is dependent on the priming with estrogen and is rapid as it occurs within 4h of P administration.

425.9

BASAL FOREBRAIN FACILITATION OF LORDOSIS BY OXYTOCIN IS BLOCKED BY A UTEROTONIC ANTAGONIST. Caldwell, J.D., Barakat, A.S., Hruby, V.J.*, Smith, D.D.* and Pedersen, C.A. BSRC and Dept. of Psychiatry, Univ. of North Carolina, Chapel Hill, NC 27599-7250

In this study we compared the effects of equal doses of utero-tonic, antidiuretic or vasopressor antagonist analogues in blocking the facilitative effects of simultaneous infusions of oxytocin (OXT). Ovariectomized estrogen-treated animals were infused through chronic bilateral cannulas in the basal forebrain with 500 ng OXT alone or in combination with a uterotonic, antidiuretic or a vasopressor antagonist analogue. OXT significantly increased lordosis responding 20 and 40 minutes after its infusion into the medial preoptic area (MPOA) or anterior hypothalamus (AH) when compared to the receptivity of normal saline vehicle infused animals. The uterotonic antagonist significantly blocked the facilitation seen after OXT. The vasopressor and antidiuretic antagonists had no effect on the OXT-induced facilitation of lordosis postures. The vasopressor antagonist facilitated sexual receptivity 90 minutes after infusion. This work confirms our earlier finding that OXT infusions into the MPOA-AH were facilitative to female sexual receptivity (Beh. Neurosci. 103:671). The facilitative effect of OXT on receptivity appears to be mediated by central uterine-like receptors which may have a separate CNS site from inhibitory vasopressor receptors.

425.10

SENSITIVITY OF BRAIN OXYTOCIN RECEPTORS TO OVARIAN STEROIDS. M. Schumacher, H. Coirini, D.W. Pfaff and B.S. McEwen. The Rockefeller University, New York.

In estrogen primed females, progesterone (P) increases the area covered by OT receptors within the ventromedial hypothalamus (VMH) . This spread of OT receptors may be a key step to the facilitation of female mating as the infusion of OT into the VMH only facilitates lordosis behavior in female rats primed with both estradiol benzoate (EB) and P. By using the OT antagonist $[d(CH_2)^5,[Tyr(Me)]^2, Thr^4, Tyr\text{-}NH_2{}^9]$-$[^{125}I]$OVT as a ligand, we found that the area covered by OT receptors within the VMH of EB and P primed females is 60% larger in the the mid-dark (D) phase than in the mid-light (L) phase of the light cycle. If the spread of OT receptors is important for the facilitation of lordosis behavior, OT should be behaviorally more efficient during the D phase when the area covered by OT receptors is greatest. The infusion of a large amount of OT (1 ug) into the third ventricle of EB+P primed unanesthetized rats facilitates lordosis behaviour with the same efficiency during the L phase and during the D phase. However, the ICV administration of a smaller amount of OT (200 ng) only facilitates the lordosis reflex during the D phase. Thus, behavioral and endocrine changes correlated with the light cycle could interact with the OT effect.

REGULATION OF AUTONOMIC FUNCTIONS V

426.1

ALTERED NEURONAL CONTENT OF CALCITONIN GENE-RELATED PEPTIDE IN LAMINAE I AND II IN THE SPONTANEOUSLY HYPERTENSIVE RAT. K. N. Westlund, D. J. DiPette, O. B. Holland, The Marine Biomedical Institute and University of Texas Medical Branch, Galveston, TX 77550

Calcitonin gene-related peptide (CGRP), produced by alternative processing of the primary transcript of the calcitonin gene, is a potent vasodilator. We have shown that dietary calcium deficiency significantly decreases the neuronal content of CGRP in laminae I and II of the dorsal horn of the spinal cord in the growing rat. To determine if the neuronal content of CGRP is altered in the spontaneously hypertensive rat (SHR), a model characterized by calcium deficiency, neuronal CGRP was localized immunocytochemically. The density of immunocytochemical staining was quantitated by computer-assisted image processing of laminae I and II of the upper thoracic spinal cord of 12-14 week old male SHR (n=4) and Wistar-Kyoto (WKy)(n=4) normotensive, control rats. The SHR had significantly decreased neuronal CGRP content compared to the WKy rats (107±5 vs 121±6 arbitrary units, $p< 0.01$). In contrast, the neuronal density of substance P, which frequently co-exists with CGRP in this neuronal population, was not different between the two groups (SHR: 91±6 vs WKy: 88±3 arbitrary units). As expected, the SHR had a significantly higher tail-cuff systolic blood pressure than the WKy rats (214±10 vs 147±9, $p<0.001$; ± SEM). In conclusion, SHR have decreased neuronal content of CGRP which appears to be specific for CGRP since substance P was not altered. Therefore, this reduction of a potent vasodilator may contribute to the hypertension. Supported by NIH grant NS11255 and Am. Heart Assoc., TX Affiliate Grants 87R-654 and 88G-663.

426.2

PARABRACHIAL EFFERENTS INFLUENCE BOTH ANGIOTENSIN AND BLOOD PRESSURE SENSITIVE NEURONS IN AREA POSTREMA. S. Papas* and A.V. Ferguson. (SPON:V.C. Abrahams) Dept.of Physiology, Queen's University, Kingston, Canada K7L 3N6

The area postrema (AP) is a circumventricular organ which has been strongly implicated in autonomic regulation. Previous electrophysiological studies have characterized subpopulations of AP neurons specifically influenced by systemic angiotensin-II (AII) (AII-sensitive), or by changes in blood pressure (BP-sensitive). We have also established a variety of orthodromic influences on rat AP neurons from the parabrachial nucleus (PBN). The present investigation was undertaken to determine any functional relationship between these groups of AP neurons and the orthodromic inputs from the PBN. A stimulating electrode was positioned in the PBN of anaesthetized Sprague Dawley rats. Single unit activity was recorded in the AP and peristimulus histograms used to determine initial effects of PBN stimulation on a total of 127 AP neurons. Thirty of these cells were also classified as being either AII-sensitive (responding specifically to i.v. AII) or BP-sensitive (responding specifically to changes in BP). Neurons responding differently to AII and BP changes were placed in both the AII- and BP-sensitive groups. Twenty-five percent of AII-sensitive cells (n=12) were excited by PBN stimulation (latency 20-40 ms, duration 10-340 ms) while 8% showed an inhibitory response (latency 80 ms, duration 110 ms). Of the 13 cells determined to be BP-sensitive, an equal number (23%) exhibited excitatory (latency 20-40 ms, duration 10-40 ms) and inhibitory (latency 10-80 ms, duration 110-160 ms) responses. In both AII- and BP-sensitive groups, remaining neurons were unaffected by PBN stimulation. Nine cells were not influenced by AII or BP changes. Of these neurons (78%) were unaffected by PBN stimulation, 11% were excited (latency 8 ms, duration 10 ms) and 11% were inhibited (latency 8 ms, duration 60 ms). These data suggest the existence of a variety of PBN influences on both AII and BP sensitive neurons in the AP. They indicate however that there is no differential PBN innervation of these two functionally separate groups of AP neurons.

426.3

RESPONSES OF RAPHESPINAL NEURONS TO CORONARY ARTERY OCCLUSION. **A.R. Evans* and R.W. Blair. Dept. Physiol., Univ. Okla. Hlth. Sci. Ctr, Oklahoma City, OK, 73190.**

The excitatory response of spinothalamic and spinoreticular neurons to coronary artery occlusion (CAO) can be inhibited by stimulation of the raphe nuclei. The purpose of this study was to determine whether raphespinal neurons (RAS) respond to separate CAO of the left anterior descending (LAD) and circumflex (CX) coronary arteries. Extracellular potentials were recorded from RAS in 20 α-chloralose-anesthetized cats. Twenty-three RAS were examined for responses to CAO (duration 35 s) of the LAD and/or CX arteries. Thirteen responses out of 37 occlusions were observed. Ten responses were inhibitory; neuronal activity decreased from 13.7 ± 5.9 to 9.6 ± 4.8 spikes/s for occlusion of CX (N=6) and from 13.4 ± 9.6 to 9.6 ± 7.9 spikes/s for occlusion of LAD (N=4). Three RAS were excited by CAO. Of the 14 RAS tested for responses to CAO of both arteries, 3 exhibited qualitatively different responses to CAO of each artery, and 11 showed similar responses. Results indicate that 1) some RAS are responsive to CAO, 2) the primary response is inhibition, and 3) some RAS are differentially responsive to CAO of separate arteries. (Supported by grant OK-87-G1 from Okla. Affiliate of AHA, and NIH grant HL29618).

426.4

A ROLE FOR SYMPATHOEXCITATORY VENTROLATERAL MEDULLARY NEURONS IN THE PRESSOR REFLEX TO MUSCULAR CONTRACTION. R.M. Bauer, G.A. Iwamoto and T.G. Waldrop, Departments of Physiology & Biophysics and Veterinary Biosciences, Univ. of Illinois, Urbana, IL 61801

Studies from this laboratory have shown that the pressor reflex to muscular contraction (MC) is attenuated following microinjections of an excitatory amino acid antagonist into the ventrolateral medulla (VLM) and the firing patterns of VLM neurons are altered during MC. The purpose of the present study was to determine if the firing patterns of VLM neurons which are altered during MC show a temporal correlation with peaks in sympathetic discharge (SND). In anesthetized cats, stimulation of L7 and S1 ventral roots induced hind limb MC. Extracellular single unit activity was recorded from 45 VLM neurons with microelectrodes positioned 1-3 mm rostral and 4 mm lateral to obex and 4.5-5.5 mm below the dorsal medullary surface. Temporal relationships between VLM units and SND were examined at rest. Computer averages of SND demonstrated that bursts of SND slow wave activity occurred 106±6 ms after unit discharge in 26 VLM neurons. MC elicited 70% or greater increases in firing frequency in 20 of 26 VLM neurons which showed temporal correlations with SND. Similar results were observed in baroreceptor intact and barodenervated cats. These results suggest that 1) MC evokes increased firing rates in VLM neurons related to SND 2) VLM neurons which may modulate sympathetic drive participate in the pressor reflex to MC.

426.5

CYTOSKELETAL PROTEINS IN POSTGANGLIONIC NEURONS AND SIF CELLS IN THE CARDIAC PARASYMPATHETIC GANGLION OF NECTURUS. T.W. McKeon and R.L. Parsons. Department of Anatomy and Neurobiology, University of Vermont, Burlington, VT 05405.

Two cells types are present in the cardiac ganglion of Necturus maculosus (mudpuppy): postganglionic parasympathetic neurons (principal cells) and small-intensely-fluorescent (SIF) cells. Principal cells have one major process and are considered "adendritic" whereas SIF cells have several processes. The present study was done to determine whether processes of these cell types contained axonal or dendritic cytoskeletal proteins. Control and deafferented cardiac ganglia were labelled using immunofluorescence techniques with antisera for either the high molecular weight forms of microtubule associated protein-2 (AP14; a gift from L.I. Binder) or for the phosphorylated form of the H and M subunits of neurofilament protein (SMI31). AP14 has been shown previously to label cell bodies and dendrites, whereas SMI31 preferentially labels axons. Identification of SIF cells was confirmed by double labeling with a serotonin antibody. In control and deafferented cardiac ganglia, principal cell somas and several hundred microns of their processes were labeled with AP14, but SIF cells and processes did not label. SMI31 labeled many axons in the cardiac ganglion but did not label the principal cell somas or their proximal processes. Following deafferentation, principal cell somas and proximal processes labeled with SMI31. SIF cells and their processes were not labelled with SMI31 in control or deafferented ganglia. These results indicate (1) SIF cells in the mudpuppy cardiac ganglion do not contain the same cytoskeletal proteins as the principal cells, (2) the proximal part of the principal cell process contains dendrite-like proteins, and (3) deafferentation promotes expression of phosphorylated forms of neurofilaments in parasympathetic postganglionic neurons. Supported by PHS NS 23978 and NS 25973.

426.6

HYPOTHALAMIC-MEDIATED WITHDRAWAL OF SYMPATHETIC TONE IN RAT MUSCLE MICROCIRCULATION. R.W. Stremel*, J.W. Brock* and I.G. Joshua* (SPON: A.E. Jimenez). Dept. of Physiol. & Biophys., Univ. of Louisville, Louisville, KY 40292.

Activation of the posterior hypothalamus (PH) of the rat induces locomotion, tachycardia, hypertension, hyperventilation, and vasodilation within striated muscle; changes consistent with activating the "central command" for exercise. The purpose of this study was to determine the mechanism by which PH activation induces this microvascular dilation. Male Sprague-Dawley rats (190-290 gm) were anesthetized with pentobarbital (50mg/kg, ip) and the cremaster muscle was suspended in a tissue bath of Ringers solution. Arterioles (12-23μ) were observed by television microscopy while diaphragmatic EMG activity, blood pressure and heart rate were monitored. Bicuculline Methiodide (250ng/50nl) was microinjected into the PH before and after local blockade of β-adrenergic receptors (n=4) and substance-P receptors (n=5). To eliminate reflex dilation due to respiratory changes and/or local vasodilator metabolites from locomotion during PH activation, animals (n=6) were paralyzed and ventilated. PH-induced vasodilation was blocked only by local addition of 6-OHDA, eliminating changes in α-adrenergic activity (n=6). Thus the mechanism for the vasodilatory response to the "central command" for exercise in the rat is due to the withdrawal of sympathetic tone from the microcirculation. Supported by Kentucky and Amer. Heart Assoc.

426.7

MATURATION OF CORRELATIONS BETWEEN PHYSIOLOGICAL MEASURES ACROSS SLEEP-WAKING STATES IN NORMAL INFANTS. V.L. Schechtman, R.M. Harper and K.A. Kluge*. Brain Research Institute and Department of Anatomy and Cell Biology, UCLA, Los Angeles, CA 90024.

Coordination between physiological measures has been suggested to be an index of physiologic maturity in infants. We examined the developmental patterns of correlations between physiological measures in full-term infants over the first 6 mo of life. Twelve-hr physiological recordings were obtained from 23 normal full-term infants at 1 wk and at 1, 2, 3, 4, and 6 mo of age. Each minute of data was classified as quiet sleep, rapid eye movement (REM) sleep, or waking by two trained observers. Pearson's r was used to assess the minute-by-minute correlations between heart rate and respiratory rate, heart rate and respiratory variability, and respiratory rate and respiratory variability during each sleep-waking state in each recording. The maturational patterns of the correlations were examined visually and assessed by 2-way (age x state) analysis of variance. Different developmental patterns were observed in each sleep-waking state. In quiet sleep, the correlations weakened over the first month of life, increased from 1 to 3 mo, then plateaued. In REM sleep, correlations tended to become stronger from birth to 3 mo, then correlations between heart rate and respiratory measures decreased while respiratory rate by respiratory variability continued to become more strongly correlated through 6 mo. During waking, all correlations increased from birth to 2 mo and decreased from 2 to 4 mo. The correlations between each of the three pairs of measures showed significant age x state interactions. The different developmental patterns suggest that over the first 6 mo of life, the coupling of physiological systems proceeds differently in each sleep-waking state. Supported by HD22506 and HD22695.

426.8

TRIGEMINAL PROJECTIONS FROM THE UPPER RESPIRATORY TRACT IN THE MUSKRAT. W. M. Panneton. Dept. of Anatomy & Neurobiol., St. Louis Univ. Sch. of Med., St. Louis, MO 63104.

The upper respiratory tract (URT) serves as the first line of defense against inhaled irritants and is innervated by the ethmoidal, glossopharyngeal (IX), and superior laryngeal (SLN) nerves. The primary afferent projections of these nerves to the trigeminal sensory complex were studied using the transganglionic transport of a mix of HRP solutions injected into the respective peripheral nerves.

The ethmoidal nerve projected densely into ventrolateral laminae I&II and V of the rostral medullary dorsal horn (MDH), the ventral paratrigeminal nucleus (vPara V), and ventromedial parts of the subnuclei interpolaris (TrSi), oralis (TrSo), and the principal nucleus (PV). A more sparse projection to the dorsomedial TrSo also was seen. The pharyngeal branch of IX sends dense projections to laminae I&V of the MDH and the ventral and dorsal ParaV and sparse projections to TrSi and dorsal parts of the TrSo and PV. The SLN also projects to lamina I&V of the MDH and the ventral and dorsal parts of the ParaV; a sparse projection to the medial edge of TrSi was noted. In addition, the nucleus solitarius was labeled in SLN and IX cases, and the reticular formation in all cases.

Thus, afferent fibers from the URT have convergent projections to lamina I&V of the MDH and the ParaV; secondary neurons in these areas may be important for the cardiorespiratory adjustments seen after URT stimulation. Supported by NIH: HL38471.

426.9

RESPIRATORY EFFECTS OF ENDOTHELIN 3 IN CONSCIOUS RATS AFTER INTRACEREBROVENTRICULAR (icv) ADMINISTRATION. S. Vonhof, G. Feuerstein and A.- L. Sirén (SPON: T. P. Jacobs), Dept. Neurology, USUHS, Bethesda, MD 20814

Binding sites for the endothelium derived peptide, endothelin, were recently described in the rat brain. Moreover, modifications of cardiovascular functions were reported upon icv administration. In order to investigate the respiratory effects of endothelin icv, we measured changes of ventilation rate (VR) and relative tidal volume (rV_T) in conscious, unrestrained Sprague-Dawley rats after icv doses of endothelin 3. Prior to the experiment, guide cannulas were implanted over the right parietal cortex (AP -0.8, L -1.2) under halothane anesthesia. The animals were allowed to recover for at least 24 hours. VR and rV_T were measured in the Oxymax 85 system (Columbus Instr.) after icv injections of 0.1, 0.3, 1, and 2 nmoles/kg doses of endothelin 3 in a volume of 15 µl. Curve integrals of the changes were calculated and used for statistical evaluation. While VR in all groups was not significantly different from control (saline), rV_T increased dose dependently by 58%, 122%, 263% ($p< .005$), and 319% ($p<.005$) compared to saline, leading to a dose dependent increase of the relative ventilatory minute volume of 48%, 214%, 328% and 482% ($p<.05$). The results indicate that endothelin stimulates ventilation and may imply a role for endothelin in the central cardioventilatory control.

426.10

MEDULLARY CONTROL OF BRONCHIAL SMOOTH MUSCLE TONE IN GUINEA PIGS: IDENTIFICATION OF A BRONCHOCONSTRICTOR SITE. J. A. Hey, M. del Prado and R. W. Chapman, Schering-Plough Corp., Bloomfield, NJ.

The role of the CNS in control of airway smooth muscle tone is poorly defined. In fact, the autonomic nuclei that govern bronchomotor tone have not been identified. To investigate the role of the medulla on airway smooth muscle tone we measured changes in pulmonary insufflation pressure (PIP) following bilateral electrical stimulation of the medulla in anesthetized ventilated guinea pigs. Blood pressure was measured to determine cardiovascular responses to CNS stimulation. Stimulation of the medulla (10s, 32 Hz, 50-700 μA) elicited an intensity-dependent increase in PIP and hypertension. The greatest increase in PIP (567 $\pm$ 19% at 700 μA) occurred at: A 1.5-3.0 mm, L 1.5 mm and V 1.0-1.2 mm from obex. The CNS-induced increase in PIP was halved by unilateral vagotomy, abolished by bilateral vagotomy and blocked in a dose-dependent fashion by the muscarinic antagonist ipratropium (1 and 10 μg/kg iv). Neither vagotomy nor ipratropium blocked the hypertension due to CNS-stimulation. In contrast, spinal section with lidocaine blocked the hypertension but not the increase in PIP. These results identify a bronchoconstrictor site in the medulla, which projects to the lungs via an efferent, cholinergic pathway. It is speculated that this medullary bronchomotor area is an important site of convergence for reflex and supramedullary bronchoconstrictor pathways in the brain.

426.11

OSCILLATIONS IN BREATHING PRESSURE OR FLOW RECORDS DUE TO AIRWAYS SMOOTH MUSCLE ACTIVITY. J. García Ramos. Lab of Neurophysiology. University of Querétaro Medical School. México.

Breathing pressure or flow oscillations can be recorded in the conscious man or anesthetized cat and dog. Since irregularities in these records may be caused by events taking place in upper air ways or extrapulmonary organs (heart, muscles) or be artifacts introduced by the recording system, experimental observations were made to exclude or control such possibilities. It was found that the irregular oscillations depend mainly from sympathetic activity: They are exxagerated by a deep inspiration, the Valsalva maneuver, carotid occlusion, cold or painful stimulation, cigarette smoking, moderate asphyxia, and emotional situations in man. Conditions when sympathetic activity are also detected in other effectors (P.G.R., superficial veins, heart rate, and nictitating membrane). Furthermore, these oscillations appear in animals with the chest open, persist after the double vagotomy, but disappear after removal of both stellate ganglia with part of the thoracic chain. It is inferred that these oscillations represent physiological activity and it is related to events occurring in bronchial smooth muscle. That activity depends from sympathetic action.

426.12

MECHANISMS OF THE ACTION OF ACETYLCHOLINE ON GLOMERULAR ULTRAFILTRATION IN THE RABBIT. X. LIU*(SPON: Jon M. Walro). NEUROBIOLOGY DEPT., N.E. OHIO COLLEGE OF MED., ROOTSTOWN, OH 44272

Acetylcholine was injected into the renal artery to examine its effect on the glomerular ultrafiltration and the renal autoregulation in rabbits. First, acetylcholine injection produced variable changes: the renal blood flow(REF) increased greatly(40%). While the ultrafiltration coefficient(Kf) decreased dramatically(38%). Both afferent and efferent arteriolar resistance(Ra & Re) decreased. Because of these offsetting effects, Glomerular filtration rate(GFR) was unchanged. Then MnCl2 was injected and the contraction of mesangial cells was blocked. In such conditions, the injection of acetylcholine failed to reduce Kf, but RBF, RA, RE had the similar changes as before. Moreover, GFR increased significantly (30%). These data gave direct demenstration that Kf is a key factor of dissociation between autoregulation of renal blood flow and glomerular filtration rate.

EPILEPSY: BENZODIAZEPINES AND INHIBITORY AMINO ACIDS

427.1

CENTRAL BENZODIAZEPINE RECEPTOR CHANGES IN THE p,p'-DDT MYOCLONIC/EPILEPTIC RAT HIPPOCAMPUS. R.R.Trifiletti and M.R.Pranzatelli. Departments of Neurology and Pediatrics, Columbia University, New York,NY 10032.

Certain benzodiazepines (BDZs) have anti-myoclonic properties in human myoclonic disorders and in the p,p'-DDT myoclonic/epileptic models in the rat. To test the hypothesis that BDZ receptor abnormalities may contribute to the neurotoxicity of p,p'-DDT, we measured central BDZ receptors in adult rats given 1gm p,p'-DDT by orogastric intubation using in vitro [^{3}H]-flunitrazepam binding. The hippocampus was chosen for initial studies as it contains a mixture of BDZ receptor subtypes. Animals treated with p,p'-DDT displayed a 23% ($P<0.05$) increase in number of BZD sites as compared to controls. Pretreatment of animals with intracisternal 5,7-dihydroxytryptamine (DHT), which markedly reduces the potency of p,p'-DDT at producing myoclonus and convulsions in the rat, abolishes the receptor number increase observed with p,p'-DDT treatment alone. The effect of p,p'-DDT does not appear to alter the ratio of BZ1 and BZ2 sites as defined by displacment with CL 218,872.

427.2

THE BENZODIAZEPINE (BZD) RECEPTOR IN CULTURED ASTROCYTES FROM GENETICALLY EPILEPSY-PRONE RATS (GEPR-9). M.D. Norenberg and I. Ducis* (SPON: R. Rotundo). Lab. of Neuropathology, Veterans Adm. Med. Ctr. and Univ. Miami Sch. Med./Jackson Memorial Hospital, Miami FL, 33101

The BZD receptor has been strongly implicated in epileptogenesis (Paul and Skulnick, Science 202: 892, 1978; McNamara et al, Proc Natl Acad Sci 77:3029, 1980). It has generally been assumed that this receptor is the neuronal one which is associated with the the GABA-chloride ionophore complex. However, astrocytes also possess BZD receptors (Bender and Hertz, Brain Res 341: 41, 1985) although they are of the peripheral-type. It is thus possible that observed changes in BZD receptors in various models of epilepsy may reflect alterations in the astrocyte receptor. Since astrocytes have long been implicated in epileptogenesis (Norenberg et al, The Biochemical Pathology of Astrocytes, Alan R. Liss, 1988), we investigated the state of astrocytic BZD receptors in culture. Astrocytes were obtained from cortices of GEPR-9 neonates. After 2 weeks, the cultures were treated with dibutyryl cyclic AMP (an agent that induces maturation in astrocytes). Scatchard analysis of ^{3}H-Ro5-4864 (1-100 nM) binding to astrocyte homogenates showed a 34% decrease in BZD receptor number ($p<0.05$) as compared to controls. No significant changes in affinity were found. Our findings indicate that a significant reduction in peripheral-type BZD receptors occurs in GEPR-9 astrocytes. Furthermore, these observations suggest that BZD receptor changes described in other epilepsy models may also occur in astrocytes.

427.3

GLIAL GABA UPTAKE INHIBITORS: ANTICONVULSANT ACTIVITY IN CHEMICAL SEIZURE MODELS IN RATS. S.E. Gonsalves, B. Twitchell*, R.E. Harbaugh*, P. Krogsgaard-Larsen* and A. Schousboe*. Depts. of Surg. and Pharmacol., Dartmouth Med. Sch., Hanover, NH 03756 and PharmaBiotec Res. Ctr., Copenhagen, Denmark.

Evaluation of glial GABA uptake inhibitors as anticonvulsants has been hampered by their inability to cross the blood-brain barrier. We compared the antiseizure properties and neurotoxicity of intracerebroventricularly (i.c.v.) injected 5,6,7,8-tetrahydro-4H-isoxazolo[4,5-*c*]azepin-3-ol (THAO) and 4,5,6,7-tetrahydroisoxazolo[4,5-*c*]pyridin-3-ol (THPO) in acute models of chemoconvulsion in rats. Maximal tonic-clonic seizures were elicited by bolus i.v. injection of pentylenetetrazol (PTZ; 25 mg/kg) or isonicotinic acid hydrazide (INH; 822 mg/kg) 30 min after THAO or THPO (100-750 µg, i.c.v.). Seizure thresholds were evaluated using timed i.v. infusions of PTZ (10.2 mg/min). THAO and THPO increased the latency to INH seizures and blocked the extensor component of maximal PTZ seizures but did not increase PTZ seizure thresholds. THAO produced fewer deficits in motor function than did THPO. The ability of THPO and THAO to suppress maximal convulsions but not generalized minor seizures suggests that the compounds block seizure spread. (Support: American Health Assistance Foundation, Pfizer, Inc., and NATO)

427.4

BICUCULLINE KINDLING IN RATS INCREASES GAD ACTIVITY IN THE NIGROTECTAL TARGET AREA. M. Garnett*, S.E. Bachust & K. Gale (SPON: J. A. Childs), Dept. of Pharmacology, Georgetown Univ. Sch. Medicine, Wash. D.C., 20007; and †Maryland Psychiat. Res. Ctr., Baltimore, MD 21228.

The integrity of the deep layers of the superior colliculus is required for protection against maximal electroshock seizures conferred by intranigral application of the GABA agonist muscimol[1], suggesting that the GABAergic nigrotectal projection may mediate this effect. We have investigated whether this projection is involved in regulating seizure susceptibility by measuring tectal GAD activity after kindling.

Adult male Sprague-Dawley rats were 'kindled' by daily treatments (for 14 days) with bicuculline (BIC)(starting at 2 mg/kg s.c. per day and reducing the dose by 0.5 mg/kg every 4-5 days). After this period, the threshold convulsive dose of BIC was <50% of that required in non-kindled (saline-treated) controls. Ten days after the last BIC treatment, the rats were sacrificed, and the superior colliculus was divided into the deep and superficial layers, bilaterally. These tissues were assayed radioenzymatically[2] for GAD activity, and values for bilateral tissues averaged for individual rats.

GAD activity was significantly increased in the deep layers of superior colliculus, where nigrotectal fibers project, in BIC-kindled rats (.455 ± .013 µmol/mg protein/hr) relative to non-kindled controls (.379 ± .016) ($p<.001$). However, there was no significant difference in GAD activity between superficial layers of superior colliculus in kindled (.687 ± .022) vs. non-kindled (.666 ± .019) rats ($p>.10$).

Thus, GAD activity is augmented specifically in the nigrotectal target area, and not in the superficial layers of superior colliculus, following BIC-kindling. These results support the hypothesis that reduced inhibition of nigrotectal activity by substantia nigra, hence elevated tectal GAD activity, is a concomitant of sensitization to BIC-induced convulsions. [1]Garant & Gale, Exp. Neurol. 97:143,1987. [2]Sims & Pitts, J. Neurochem. 17.1607, 1970.

427.5

GABA AND GLUTAMATE RECEPTORS IN SUBSTANTIA NIGRA CONTROL SEIZURES EVOKED FROM AREA TEMPESTAS R. Maggio* and K. Gale (SPON: R. Meloni) Department of Pharmacology, Georgetown University Medical Center, Washington, D. C. 20007

We studied the functional relationship between the substantia nigra (SN), and the area tempestas (AT), an epileptogenic site in the deep prepiriform cortex from which bilateral motor seizures are elicited with unilateral microinjections of pmole doses of bicuculline. Bilateral microinjections of the $GABA_A$ agonist muscimol (219 pmol) or of the competitive NMDA-receptor antagonist, 2-amino-7-phosphonoheptanoic acid (AP7, 50 nmol), protected against convulsions evoked by the unilateral focal microinjection of bicuculline methiodide (118 pmol) into AT in freely moving rats. When muscimol was applied to the SN unilaterally (either ipsilateral or contralateral to the AT injection), no seizure protection was obtained. Bilateral intranigral application of morphine sulfate (26 or 53 nmol) did not protect against seizures elicited from AT, although it induced stereotyped sniffing and gnawing behavior similar to that evoked by intranigral muscimol and AP7.

Our data indicate that the bilateral convulsions evoked from the AT of one hemisphere are susceptible to suppression by augmentation of GABA transmission or blockade of NMDA receptors in SN, but only when the inhibition occurs in the SN of both hemispheres. This suggests that the seizure suppressant action evoked from SN is exerted during the course of propagation of the seizure, once it has spread bilaterally.

427.6

$GABA_A$ RECEPTOR CLONES FROM SEIZURE PRONE AND RESISTANT MICE. D.R. Burt, J.C.R. Fernando, P. Kofuji*, J.B. Wang* and D.K. Overman*. Dept. of Pharmacology, Univ. of Maryland School of Medicine, Baltimore, MD 21201.

DBA/2J inbred mice are prone to audiogenic seizures as juveniles, while C57BL/6J inbred mice are resistant to seizures at any age. Evidence has suggested that differences in the two strains' seizure susceptibilities arise in part from differences in the structure or regulation of their inhibitory $GABA_A$ receptors. To explore this possibility further, we have constructed size-selected cDNA libraries in lambda gt10 from whole brains of both strains and have screened them (c. 2 x 10^6 independent recombinants each) at low stringency with cDNA probes based on published bovine $GABA_A$ receptor sequences (Schofield et al., Nature, 328:221, 1987). This work, still in progress, has yielded over 300 positives, which, by differential cross hybridization, separate into a number of families of distinct but related sequences. End sequencing of the shortest clones of each of two families to date has indicated that these families include the mouse homologs of the bovine alpha-3 subunit and a bovine beta subunit (Levitan et al., Nature, 335:76, 1988). The nature of the strain differences, if any, in these clones' sequences or expression, and their possible relationship to the allelic differences we reported last year (Fernando and Burt, Soc. Neurosci. Abstr. 14:1035, 1988), is under investigation. (Supported by USPHS grant NS25525.)

427.7

ASSESSMENT OF GABA UPTAKE AND GLUTAMIC ACID DECARBOXYLASE (GAD) ACTIVITY IN THE GENETICALLY EPILEPSY-PRONE RAT (GEPR) BRAIN. R.A. Browning, M. Marcinczyk*, and P.C. Jobe. Southern Ill. Univ. Sch. Med., Carbondale, IL 62901 and Univ. Ill. Coll. Med., Peoria, IL, 61656.

Microinjections of GABA agonists into the inferior colliculus (IC) inhibit audiogenic seizures in severe seizure GEPRs (GEPR-9s) and iontophoretic application of GABA to cells in the IC causes less inhibition in GEPRs than in normal rats (Faingold, Gen Pharmacol. 19 331,1988). On the other hand, the GEPR-9 IC has increased numbers of GAD-immunoreactive (GABAergic) neurons compared to normal rats (Roberts et al., Brain Res. 361 324,1985). To further assess GABAergic neurotransmission in the GEPR-9s, we compared high affinity GABA uptake and GAD activity in the IC (and other regions implicated in audiogenic seizures) of GEPR-9 and normal rats. High affinity uptake of ^{3}H-GABA was measured in resuspended P_2 synaptosomes isolated from specific brain regions and GAD activity was determined in homogenates of specific brain regions by measuring the rate of decarboxylation of ^{14}C-glutamic acid. GABA uptake was found to be increased (16%, P<.05) in synaptosomes isolated from the IC of GEPR-9s when compared to normal rats, but was not changed in other brain regions (pontine reticular formation, substantia nigra, or hippocampus). GAD activity was increased (23%, P<.001) in the GEPR-9 hippocampus, but was not altered in the IC or other regions examined. The uptake findings are consistent with the increase in the number of GABAergic neurons in the IC of GEPRs, but the lack of a difference in GAD activity questions the functional significance of the increased neurons. Additional studies will be needed to determine if these differences contribute to the seizure prone state of GEPR-9s. Supported by The Deafness Research Foundation.

427.8

LONG-TERM CHANGES IN SENSITIVITY TO GABA IN DORSAL RAPHE NEURONS FOLLOWING AMYGDALOID KINDLING. T.D. Hernandez, J.B. Rosen, C. Heninger and D.W. Gallager. Dept. Psychiatry, Yale Univ. Sch. Medicine, New Haven, CT 06508

Work from our laboratory has shown that chronic exposure to diazepam produces a decrease in bicuculline seizure threshold which occurs in parallel with the progressive development of subsensitivity to GABA in dorsal raphe neurons. In addition, work from other laboratories have reported that manipulations within the midbrain raphe region affect amygdala kindled seizure threshold and expression. The present studies were undertaken to evaluate GABA sensitivity in dorsal raphe neurons following amygdala kindled seizures.

Amygdala kindling decreased bicuculline seizure threshold. Dorsal raphe lesions prior to amygdala kindling significantly lowered kindled seizure threshold. Dorsal raphe neurons of amygdala kindled rats exhibited significant subsensitivity to GABA, as measured electrophysiologically 3-4 weeks after the last Stage 5 seizure. Amygdala stimulation with currents that did not produce kindled seizures did not produce subsensitivity to GABA. The subsensitivity observed after kindling was equivalent in magnitude to that observed following chronic diazepam treatment. However, chronic diazepam exposure in fully kindled rats did not further decrease the sensitivity of dorsal raphe neurons to GABA. Additionally, while subsensitivity to GABA was reversed by bath application of Ro 15-1788 in chronic diazepam treated rats, the benzodiazepine antagonist had no effect on GABA subsensitivitiy in fully kindled rats. These findings suggest that GABAergic sensitivity within the dorsal raphe might reflect long-term neuronal changes associated with kindled seizures. These data also suggest that GABA sensitivity changes following chronic diazepam may involve different mechanisms than those observed after amygdala kindling.

427.9

GENETIC VITAMIN B_6 DEPENDENCY AS A DETERMINANT OF EPILEPTIC DIATHESIS. S. Dolina*, A. Keller*, A. Kozak*, (SPON: S.J. Shefchyk) Department of Biochemistry and Molecular Biology, University of Manitoba, Winnipeg, MB, Canada R3E 0W3; Ben-Gurion Univ. of Negev, Israel; Weizmann Institute of Sciences, Israel.

Genetic vitamin B_6 (B_6) dependency/relative deficiency is considered as a significant determinant of epileptic diathesis and starting point for expanding neurotransmitter disorders. The experiments were carried out in specially developed syngeneic epilepsy-prone (EP) and epilepsy-resistant (ER) substrains of BALB/c mice. A group of EP females was treated with B_6 (10 mg/kg in drinking water) throughout the entire period of pregnancy and lactation; then offsprings received the same treatment until their maturity. The treated EP mice differed from nontreated in the following way:

1) High tolerance to pentamethylentetrazol (PTZ) in a wide range of doses; B_6 withdrawal followed by decreased tolerance to PTZ.
2) Normalized number of GABA-neurons in inferior colliculi and in motor cortex, genetically increased in EP mice.
3) Normalized phosphatidylinositol (PI) level in brain stem plasma membranes with the same tendency in cerebellar ones. The membranes of both structures are characterized by increase PI level in non-treated EP mice.
4) Phosphatidylcholine accumulation in brain stem and cerebellar membranes.

Clinical application of longterm B_6 treatment (90 mg/day) in an infant with uncontrolled behaviour and nightmares (family history of epilepsy in both parents) have been followed by disappearance of epileptic EEG patterns and behavioral normalization. Early correction of B_6 dependency as an inborn error of metabolism is suggested as a new approach to the treatment of epilepsy at very early stages of the disease as well as to the prevention of the build-up of epileptic diathesis in cases of high family risk.

427.10

GLYCINE POTENTIATES DIAZEPAM AND SODIUM DIVALPROATE IN SUBCUTANEOUS PENTYLENETETRAZOL SEIZURES, S.L. Peterson Dept. of Medical Pharmacology and Toxicology, Texas A&M University, College Station, Texas 77843.

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

Minimal threshold seizures were induced by a s.c. injection of a 75 mg/kg dose of pentylenetetrazol. A clonic convulsion consisted of a 5 sec period of clonic spasms. Statistical significance between anticonvulsant dose-response curves with and without glycine were determined by nonlinear regression analysis.

Glycine (40 mmol/kg, p.o.) administered 1, 2, 4 or 8 hours before the seizure test had no effect on seizure response. However, glycine (40 mmol/kg, p.o.) significantly reduced the ED_{50} of diazepam (1.26 to 0.76 mg/kg, i.p.) and sodium divalproate (307.5 to 240.4 mg/kg, i.p.) without affecting the neurotoxicity induced by either drug. Glycine did not significantly affect the ED_{50} of phenobarbital or ethosuximide.

These findings do not support proposed glycine-GABA interactions and suggest that glycine acts by a variety of independent mechanisms to potentiate anticonvulsants. (Supported by NIH Grant 24566)

427.11

DETERMINATION OF LORAZEPAM IN RAT BRAIN USING COMBINED TECHNIQUE OF LIQUID-LIQUID EXTRACTION, SOLID-PHASE EXTRACTION AND HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY (HPLC). S. Gunawan, N.Y. Walton and D.M. Treiman (SPON: A.V. Delgado-Escueta). Neurology and Research Services, VAMC, Los Angeles, CA 90073 and Dept. of Neurology, UCLA Sch. of Med., Los Angeles, CA 90024.

Lorazepam (LZP), a 1,4-benzodiazepine is used in the treatment of status epilepticus. In order to study the pharmacokinetics of LZP entry into brain, we developed a method for quantitation of LZP in rat brain using HPLC. LZP was extracted from 100 mg of brain tissue, which was homogenized by ultrasonic disruption in 1 ml 1.0 M Tris-buffer (pH=10.5). Type VIII alkaline protease was added to the homogenate and the mixture was incubated for 1 hr at 50°C. Toluene was used to extract the drug and internal standard. Chlordiazepoxide (CDX) served as internal standard. The organic phase was dried and the residue redissolved in methanol and washed against iso-octane. The resulting extract was applied into a 1 ml C18-Bond Elute column and washed with 2 ml of water. LZP and CDX were eluted with 250 ul of 70% methanol solution. LZP and CDX were separated using a Rainin C18-microsorb column (250 X 4.6 mm ID) and monitored by a UV detector set at 240 nm. The mobile phase was methanol/0.025 M Na_2HPO_4 (66/34, V/V) and the flow-rate was maintained at 1 ml/min. LZP eluted at 7.9 min followed by CDX at 10.0 min. Plots of peak height ratios versus concentrations were linear over the range of 20-200 ng LZP.

427.12

DISTRIBUTION OF VALPROIC ACID IN THE RAT BRAIN Thomas J. Hoeppner, Rush Medical College, Chicago, Ill. 60612

Valproic acid (VPA) is one of the most effective anticonvulsants available, yet its mechanism of action is unknown. The present studies were undertaken in the belief that determining the distribution of VPA in the brain would provide a guide to further studies to identify the specific mechanisms responsible for its action.

Radiolabeled valproic acid (as the sodium salt) was injected intravenously into anesthetized rats. One hour later (the time of maximal effectiveness of the drug) the animals were sacrificed and the brain removed and frozen. The distribution of VPA was determined in thaw-mounted brain sections using contact autoradiography. VPA concentrated in the glomerular layer of the olfactory bulb with almost no accumulation elswhere in the brain. VPA distribution was restricted to the medial and dorsal portions of the bulb (areas with notable concentration of GABA terminals and peripheral benzodiazepam receptors).

We have also compared the distribution of VPA in animals with and without perfusion with saline and fixative. Perfusion clears the isotope from the vasculature (where it binds to albumin), but leaves the high concentration in the olfactory bulbs.

The glomerular layer of the olfactory bulb may contain the long sought binding sites for VPA.

427.13

EFFECTS OF $GABA_B$-RELATED DRUGS IN TWO TYPES OF PAROXYSMAL ACTIVITY. S. Brailowsky, S. Badillo*, S. Meneses*[1] and G. Di Scala*[2]. Instituto de Fisiología Celular, U.N.A.M., México 04510 D.F., [1]Facultad de Psicología, U.N.A.M., [2]Centre de Neurochimie, C.N.R.S., Strasbourg, France.

In previous experiments, we have shown that chronic intracortical infusions of GABA induce, upon withdrawal, the appearance of spontaneous paroxysmal activity. This "GABA-withdrawal syndrome (GWS)", found in rats and baboons, has been proposed as a new model of partial epilepsy (Brain Res., 442:175, 1988).

Interested in elucidating the contribution of $GABA_B$ receptors in this phenomenon, we applied phaclofen, a new $GABA_B$ antagonist, in rats showing a GWS. Intracortical microinjections (0.2 ul, 10 nM) of phaclofen failed to show antiepileptic effects, while being effective in diminishing or arresting the paroxysmal activity induced by acute, intracortical injection of baclofen (0.2 ul, 10 nM). Phaclofen, applied unilaterally into the somatomotor cortex of normal, non-treated rats, had no behavioral (elevated beam walking test) or electrographic effects.

These data suggest that $GABA_B$ receptors have little or no involvement in the GWS, and confirm previous reports on the epileptogenic effects of intracerebrally administered baclofen. A laminar analysis of the GWS and of the baclofen-induced effects would be necessary to determine whether the two types of paroxysmal activity have a relationship with $GABA_B$ receptor distribution in the cortex.

427.14

STEROID REGULATION OF HIPPOCAMPAL NERVE CELL GROWTH IN CULTURE. B.R. Johnson* and R.E. Brinton, School of Pharmacy, University of Southern California, Los Angeles, CA 90033.

Steroid factors play a critical role in the development and adapation of the nervous system (McEwen and Brinton, 1987). We have recently found that the metabolite steroid, 5 α-pregnan-3α-ol-20-one (DHP) decreases nerve cell excitability in a GABA dependent manner and protects against chemically induced seizures (Gee et al., 1988). The purpose of this study was to determine the effect of the steroid DHP, which decreases nerve cell excitability, and 17 β-estradiol, which increases excitability, upon nerve cell growth in culture. Hippocampal nerve cells were cultured from E18 rat pups and seeded in serum containing media. Following attachment, media was exchanged for serum free. Nerve cells treated with 30 nM DHP showed a rapid response to the steroid. Within 15 min following DHP exposure the number of microspikes along the neuritic shaft was reduced by 20% and by 30 min was reduced by 80%. In addition to the decrease in microspike number, the total area of the growth cone was reduced. The length of the neuritic extension was also decreased but is probably reflective of the reduced area of the growth cone. In contrast to the effect of DHP, estradiol (30 nM), induced microspike formation along the neuritic shaft. This effect was maximally apparent within 30 min. Further characterization studies are in progress.

NEUROENDOCRINE REGULATION: NEUROHYPOPHYSIAL PEPTIDES

428.1

STIMULATORY EFFECT OF PROLACTIN ON OXYTOCIN SECRETION IN THE LACTATING RAT. W.E. Armstrong, C.E. Grosvenor, and W.R. Crowley. Depts. of Anatomy & Neurobiology, Physiology & Biophysics, and Pharmacology, University of Tennessee, Memphis, College of Medicine, Memphis, TN 38163.

In lactating rats, stimulation of dopamine (DA) receptors blocks suckling-induced oxytocin (OT) release, while blockade of DA receptors significantly increases OT release. Further studies with selective agonists and antagonists demonstrated that stimulation of the D-2 receptor with (±)-PPHT HCl completely prevented suckling-induced OT release, while a D-1 agonist, R (+)-SKF-38393 HCl, was ineffective. The selective D-2 receptor antagonist domperidone blocked the inhibitory effect of the D-2 agonist, and when given alone to nonsuckled rats, significantly stimulated OT release. To test whether DA acts within the neural lobe to suppress OT release, isolated stalk-neurointermediate lobes (sNIL) were perifused in Krebs-Ringer medium and stimulated electrically for 4 sec after 70 and 170 min of perifusion. Neither agent (1μM) affected basal or stimulated OT release, suggesting that DA does not act directly on the neural lobe to inhibit OT release. To test whether the effects of DA agents were secondary to a change in prolactin (PRL) secretion, biologically active ovine PRL (4 mg/kg), administered sc to nonsuckled, lactating rats, promptly increased plasma OT. Ovine GH was ineffective. Moreover, ovine PRL (600 ng/ml) enhanced electrically-evoked release of OT from sNIL *in vitro*. These findings suggest 1) that the effects of D-2 agonists to suppress and of D-2 antagonists to mimic suckling-induced OT release are secondary to their actions to prevent or increase, respectively, PRL release, and 2) that in lactating rats, PRL may exert a physiological effect to promote OT secretion.

428.2

Effect of short-term treatment with vasopressin (AVP) and desmopressin (DDAVP) on cerebral glucose utilization in Brattleboro (DI) rats. M.L.Terrell, E. Nermo-Lindquist*, M. Kadekaro, S. M. Kelly*, S. Freeman*, P.M.Gross# and H.M. Eisenberg. Div. of Neurosurgery, UTMB, Galveston, Tx. USA; #Dept. of Surgery, Queen's University, Kingston, Ontario, Canada.

Previous studies have shown that DI rats have high rates of glucose utilization in the neural lobe, which are not altered by chronic treatment with AVP in spite of regression of polydipsia and polyuria. To determine if this was due to the desensitization of AVP receptors, we studied with the [^{14}C]deoxyglucose method the effect of short-term treatment with AVP (5 U/kg,i.m.,twice a day) and DDAVP (100 ug/kg,ip, once a day) for two days on the activity of the hypothalamo-neurohypophysial system and related structures in DI rats (n=45). AVP and DDAVP reduced water intake, plasma osmolality and plasma Na+ concentration to the same extent. AVP decreased glucose utilization in the supraoptic n., subfornical organ, and median preoptic n., but did not alter the activity in the paraventricular n. and neural lobe. DDAVP, however, decreased glucose utilization in all these structures. These results indicate that DDAVP has a more potent action on the hypothalamo-neurohypophysial system than AVP and that the lack of response in the neural lobe from chronic treatment with AVP is not due to desensitization of AVP receptors.

428.3

BRAINSTEM ORIGINS OF SUBSTANCE P-IMMUNOREACTIVE PROJECTIONS TO NEUROSECRETORY CELL GROUPS IN THE PARAVENTRICULAR NUCLEUS. J.C. Bittencourt[1], R. Benoit[2] and P.E. Sawchenko[1]. The Salk Institute[1], San Diego, CA 92138 and Montreal Gen. Hosp.[2], Montreal, Canada.

Anatomical and pharmacological evidence suggests a role for substance P (SP) in the control of vasopressin (AVP) secretion, but the origins of SP-immunoreactive (IR) projections to the paraventricular (PVH) and supraoptic nuclei of the hypothalamus have not yet been identified. Axonal transport, immunohistochemical, and lesion approaches were used to address this issue. The results indicate that: (1). SP-IR varicosities are distributed broadly throughout the parvocellular division of the PVH, and preferentially to aspects of the magnocellular neurosecretory system in which AVP cells are concentrated. (2). Combined retrograde transport-immunohistochemical studies identified SP-IR in subsets of A1 and C1 catecholamine neurons that project to the PVH. The only other prominent brainstem contribution to the SP-IR projection arose from the laterodorsal tegmental nucleus (LDT), including some cholinergic cells. (3). Unilateral knife cuts at the level of the facial motor nuclei, which resulted in pronounced depletion of catecholaminergic varicosities in the PVH, also markedly reduced SP-IR, particularly in the magnocellular division. Coupled with the results of recent tracing studies, the results suggest that SP-IR inputs to the magnocellular system arise principally from the A1 catecholamine cell group. The LDT and C1 adrenergic cell groups appear to contribute to the innervation of the parvocellular division.

Supported by the NIH, the MRC and FAPESP (Brazil).

428.4

PROGESTERONE REGULATION OF OXYTOCIN MRNA LEVELS IN THE PARAVENTRICULAR NUCLEUS. Brooks, P.J., Caldwell, J.D., Lund, P.K.*, and Pedersen, C.A. The Curriculum in Neurobiology, Univ. of North Carolina at Chapel Hill, Chapel Hill, NC, 27599

The purpose of the present study was to determine whether exposure to late pregnancy levels of progesterone (P) regulates oxytocin (OXY) mRNA levels in the paraventricular (PVN) and supraoptic (SON) nuclei.

Ovariectomized female rats were implanted with 2mm Silastic capsules containing estradiol (E), and injected with either P (4 mg/day) or oil vehicle for 10 days. Total RNA was isolated from the PVN and SON using a small scale guanidine thiocyanate/CsCl method. Oxytocin mRNA levels were measured using a solution hybridization-RNAse protection assay. The probe was a 131 base antisense RNA transcript from linearized plasmid pGEM-OXY3c (a gift of Dr. Thomas G. Sherman, Univ. of Michigan).

No effect of P was detected in the SON. In the PVN however, P significantly decreased OXY mRNA levels relative to E alone. These results indicate that long term exposure to high levels of P, in the presence of E, decreases OXY mRNA levels in the PVN.

428.5

VASOPRESSIN SYSTEMS IN CHINESE HAMSTERS THAT HAVE DIABETES INSIPIDUS INDUCED BY MEDROXYPROGESTERONE ACETATE. H.J. De Vries*, G.J. De Vries, C.F Ferris, J. Brewer*, and J. Coe*, Neuroscience and Behavior, Univ. of Massachusetts, Amherst; Dept. of Physiology, Univ. of Massachusetts, Worcester; National Institute of Health, Hamilton, Montana.

Injections with medroxyprogesterone acetate (MPA or Depo-Provera) induce severe diabetes insipidus (DI) in Chinese Hamsters (Cricetulus griseus) that lasts for over three months. Injections with pitressin tannate temporarily eliminate the DI (Coe and Ross, 1986). We studied, therefore, whether MPA induced changes in central VP systems. Hamsters were made DI with two injections of 5 mg MPA spaced one week apart. Controls received cholesterol or saline injections. Two weeks after the first injection, controls drank 5ml per day or less whereas MPA-treated animals drank 29 mls per day or more. Animals perfused at that time showed a paraventricular (PVN) and supraoptic nucleus (SON) that stained equally well in brains of MPA-treated hamsters (n=5) and controls (n=5) in VP immunocytochemistry. Radioimmunoassay showed no statistically significant differences in AVP levels in blood, pituitary, PVN and SON (n=6 per group). As they tended to be higher in the MPA-treated animals, MPA does not seem to inactivate VP neurosecretion. (Supported by NSF grant BNS-8809799 to GJD and NIH grant NS-23557 to CFF)

428.6

DIFFERENTIAL LOCALIZATION OF ESTROGEN RECEPTORS IN VASOPRESSIN CELLS OF THE RAT BRAIN. J.F. Axelson[1] and F.W. van Leeuwen[2]. Holy Cross College[1], Worcester, MA 01610 and Netherlands Institute for Brain Research[2], Amsterdam.

Vasopressin (VP) cells in the bed nucleus of the stria terminalis (BST), medial amygdaloid nucleus (AME), supraoptic (SON) and paraventricular (PVN) nucleus are influenced by gonadal steroids. The goal of the present paper was to examine whether VP cells in the BST, AME, SON and PVN contain estrogen receptors. Brains from adult short-term castrated, colchicine-treated male rats were fixed for 8 hrs in 4% PAF and 0.5% glutaraldehyde. In the immunocytochemical double staining procedure vibratome sections were first incubated with estrogen receptor antibody (#H222, Abbott, Chicago) and stained with DAB-Ni^{+}. Following a methanol-H_2O_2 wash, sections were then incubated with anti-VP-neurophysin and stained with DAB. Parvocellular cells in the BST and AME were double stained with a blue-black nucleus (indicating the estrogen receptor) surrounded by brown cytoplasm (resulting from VP-neurophysin immunoreactivity). Our results provide the first direct anatomical evidence supporting the hypothesis that gonadal steroids' influence on parvocellular VP cells in the BST and AME is mediated directly via estrogen receptors localized in nuclei of VP neurons. We were unable to localize any estrogen receptors in magnocellular VP and oxytocin cells in the SON and PVN, suggesting that estrogen indirectly impacts magnocellular hypothalamic cells.

428.7

NEURAL LOBE EXTRACELLULAR SPACE AND ITS DEPENDENCY ON EXTRACELLULAR FLUID POTASSIUM (OR SODIUM?) AS OBSERVED AFTER ULTRA-RAPID FREEZING. M. Tian, J. Reger* and W.E. Armstrong. Dept. of Anat. Neurobiol., Univ. Tenn., Memphis, The Health Science Center, Memphis, TN 38163.

A large increase in extracellular potassium (5- 10 mM) accompanies increased neuronal activity in isolated neural lobes (Leng and Shibuki, *J. Physiol.* 392: 97, 1987). Elevated potassium levels could influence neural lobe morphology due to the ion's uptake by pituicytes, and ultimately affect stimulus-secretion coupling and/or hormone diffusion. The influence of altering the extracellular sodium/potassium ratio on neural lobe extracellular space was assessed *in vitro* by preserving fluid distribution with ultra-rapid freeze fixation.

Two groups of freshly isolated rat neural lobes were incubated for 15 min in identical oxygenated media with the exception that control medium contained 3 mM KCl, 124 mM NaCl, whereas the test medium contained 13 mM KCl, 114 mM NaCl (iso-osmotic with controls). The tissue was then rapidly frozen against a copper block, freeze-substituted in acetone-osmium and prepared for electron microscopy.

The extracellular space was dramatically reduced in the high KCl medium to ~14% of the total tissue surface area, compared with ~28% in controls. This reduction was not accompanied by a change in the average width between closely apposed processes (~23 nm in both groups), but the contribution of this narrow area to the total extracellular space was much greater in high KCl (16% vs 4% in controls). Much of this appeared due to a swelling of pituicytes and their increased enclosure of neurosecretory axons, a situation normally associated with *decreased* hormone demand (Tweedle and Hatton, *Neurosci.* 20: 241, 1987). Further studies will address the contribution of smaller ionic changes and also attempt to determine the relative influence of each ion's concentration (i.e., reduced sodium and elevated potassium) on the effect. Supported by NIH grant #NS23941 (WEA).

428.8

ESTROGEN AND PREGNANCY ALTER RAT NEUROINTERMEDIATE LOBE OXYTOCIN, VASOPRESSIN, METHIONINE ENKEPHALIN, AND DYNORPHIN. J. A. Schriefer. Department of Pharmacology, West Virginia School of Osteopathic Medicine, Lewisburg, WV 24901.

The magnocellular neurons of the hypothalamo-neurohyppophysial system (HNS) may contain opioid peptides such as methionine enkephalin (MENK) and dynorphin 1-8 (DYN) as well as oxytocin (OT) and vasopressin (AVP). The opioids may provide feedback control on OT and AVP release. If the opioids have such a functional role, the content or release of the opioids might be expected to change under conditions in which OT or AVP change. To examine this expectation, the neural lobe content and HNS release of the four peptides were determined under conditions in which OT is known to change.

Diestrus, diethylstilbesterol(DES)-treated, and day 22 pregnant rats were decapitated, the HNS excised and superfused in oxygenated Krebs buffer at 37° C. Following superfusion, neurointermediate lobes (NIL) were removed and homogenized. Peptide content of superfusates and homogenates were determined by specific RIAs. NIL OT was increased in DES-treated and pregnant rats. AVP and DYN increased in pregnant rats only. MENK content was decreased in DES-treated and pregnant rats. Release of the peptides from the HNS paralleled changes in NIL content. These data are consistent with a functional role for the co-localized opioids in the control of OT and AVP release.

Supported by NIH Grant HD 22362 and a WVSOM Intramural Research Grant.

428.9

ISOPROTERENOL ATTENUATES α_1 ADRENERGIC RECEPTOR EVOKED VASOPRESSIN (VP) RELEASE FROM PERFUSED RAT HYPOTHALAMO-NEUROHYPOPHYSEAL EXPLANTS (HNH). L.P. Renaud, R. Nissen, and D. Bichet*, Centre for Research in Neuroscience, Montreal General Hospital and Hôpital Sacré Coeur, Montreal, Canada, H3G 1A4.

Noradrenaline is both facilitatory and inhibitory to the firing of hypothalamic supraoptic neurosecretory neurones, a result of dose-dependent activation of α_1 or β adrenoceptors respectively. This study investigates the effects of α_1 vs β adrenoceptor activation on VP release from HNH. Rat HNHs were intra-arterially perfused with warmed, oxygenated artificial cerebrospinal fluid. The anterior lobe of the pituitary was removed and a suction pipette was positioned over the neurointermediate lobe to collect superfusate. Under basal conditions, samples assayed for VP by radioimmunoassay revealed baseline values of 0.5 - 11.6 pg/ml. VP levels were increased 4-13 fold when the perfusion media contained the α_1 agonist methoxamine (60 μM). In contrast, isoproterenol (60 μM) had no effect. However, addition of isoproterenol during methoxamine application attenuated the α_1 evoked response by 53 - 100%. This supports the hypothesis that β adrenoceptor activation suppresses VP release. (Supported by MRC and FRSQ).

428.10

SOMATOSTATIN-28, BUT NOT SOMATOSTATIN-14, HYPERPOLARIZES SUPRAOPTIC MAGNOCELLULAR NEURONS IN THE RAT. W.N. Raby, C.W. Bourque, R.A. Benoit and L.P. Renaud. Centre for Neuroscience Research, Montreal General Hospital and McGill University, Montreal, Quebec, Canada, H3G 1A4.

An ever increasing role is being ascribed to somatostatin (SS) as a CNS neurotransmitter. Within the rat hypothalamus, fibers displaying SS-28 like immunoreactivity are located in and around the supraoptic nucleus (SON). The inhibitory effect of SS-28 (0.1-10 μM) is most prominent on actively firing magnocellular neurons (MNCs) recorded in superfused rat hypothalamic explants. For 29/36 MNCs tested, SS-28 produced a hyperpolarization of 2 to 10 mV lasting between 2 to 5 minutes, which was sustained by a 10 to 200 % increase in conductance (n = 5). During this effect no changes were observed in the after-hyperpolarizing potential (AHP), action potential broadening, the depolarizing after-potential (DAP). No depolarizing effects were seen.

Infusions of SS-14 at similar concentrations in cells responsive to SS-28 failed to produced any change in membrane potential or conductance in 9 of 11 trials. This data suggests differential effects of somatostatin cleavage products within the rat hypothalamus. Funded by MRC of Canada.

428.11

FUNCTIONAL NEUROLOBECTOMY INDUCED BY CONTROLLED COMPRESSION OF THE PITUITARY STALK. Janos Dohanics*, G. E. Hoffman, and J. G. Verbalis (Spon: T. Plant). Depts. of Medicine & Physiology, Univ. of Pittsburgh, Pittsburgh, PA 15261.

Because selective surgical neurolobectomy is a relatively difficult procedure, we investigated an alternate method to produce selective functional neurolobectomy in rats. The pituitary stalk of anesthetized rats was compressed using a triangle-shaped wire which was introduced stereotaxically 4.0 mm caudal to the bregma in the midline and pressed against the floor of the skull for 30 sec. Water intake of rats was monitored postoperatively, and 3 weeks after surgery the rats' ability to secrete vasopressin (AVP) and oxytocin (OT) in response to 2M NaCl infusion was studied. At the end of the experiment, the neurointermediate lobes of the pituitaries were extracted for AVP and OT measurement. Additional rats were perfused and the brains immunostained for AVP-neurophysin (AVP-NP) and OT-neurophysin (OT-NP) to determine cell survival rate following the compression. Water intake of stalk compressed (SC) rats showed a characteristic triphasic response with a marked increase during the first 2d postoperatively, followed by a period of normal water intake for 1-3d and then a sustained 2-3 fold increase for the rest of the observation period. Pituitary AVP and OT content was reduced 95% in SC rats. Histologic examination of the pituitaries showed substantial degeneration in the posterior lobe, while the adenohypophysial tissue appeared to be intact. SC caused a 69% and 75% loss of AVP-NP magnocellular neurons in the supraoptic (SON) and paraventricular nuclei (PVN), respectively, while the loss of OT-NP neurons in the SON and PVN was only 30% and 32%, respectively. AVP and OT responses to iv. infusion of 2M NaCl were significantly blunted in SC rats compared to controls: $pAVP_{cont}$=2.57(pNa-141.8), r=0.68; $pAVP_{SC}$=0.06(pNa-46.3), r=0.19; pOT_{cont}=3.30(pNa-142.2), r=0.60; pOT_{SC}=0.002(pNa+5440), r=0.00. These results show that controlled compression of the pituitary stalk results in selective degeneration of the neural lobe without causing ischemic damage to the anterior pituitary, and produces marked sustained functional deficits in AVP and OT secretion. However, OT-NP neurons appear to be less significantly susceptible to damage than AVP-NP neurons following SC, suggesting the possibility of recovery of function over longer periods.

428.12

DENERVATION OF THE RAT POSTERIOR PITUITARY: VALIDATION OF A NOVEL SURGICAL APPROACH. G.B. Makara Inst. Exptl. Mod. Hung. Adac. Sci., H-1450 Budapest, Hungary.

The nerve fibers running to the posterior pituitary of the rat were interrupted without destroying of the hypothalamo-pituitary portal circulation. The pituitary stalk was compressed using a Halász-type rotating knife with a 2 mm blade (0.6 mm diameter, cutting edges at the sides, blunt tip) at 110° to the shaft. The knife was lowered in the midsagittal plane until it touched the bone at 5.3 mm behind the bregma (with the incisor bar 10° nose down), then withdrawn 0.5 mm, turned $\pm$ 45°, then repeatedly lowered 0.1 mm and turned $\pm$ 45° until friction against the basal bone surface was felt. The knife was withdrawn in the midsagittal plane. Successful operations were followed by an immediate increase in water consumption and urine output (over 80 ml/rat/day). At autopsy the continuity of the stalk could be seen and the anterior lobe appeared normal. One week after the operation Gömöri-positive neurosecretory material, neurophysin and arginine vasopressin-like immunoreactivity disappeared from the neural lobe, AVP and neurophysin piled up in the proximal part of the pituitary stalk and CRF immunoreactivity did not change appreciably in the median eminence. In a minority of rats the pituitary stalk was disrupted or the portal circulation severely damaged as shown by infarction in the anterior pituitary. Plasma corticosterone and prolactin levels increased within 15 min after surgery but returned to control levels 2 days later. In female rats the estrus cycle was temporarily disturbed after the operation. Denervation of the posterior lobe may help in studying the role of the neurohypophysial neurosecretion in various endocrine conditions
(SPON: P.M. Plotsky)

428.13

EFFECT OF CHRONIC HYPERTONICITY ON BASAL AND OSMOTICALLY STIMULATED VASOPRESSIN RELEASE. *C. Yagil and C.D. Sladek (SPON: ML Blair) University of Rochester School of Medicine, Rochester, NY 14642.

Effect of chronic hypertonicity on VP release from HNS explants was studied in explants maintained in static organ culture. The explants were positioned on a nylon mesh supported by a wire screen in individual culture wells and the dishes were maintained in a humidified incubator at 37°C under 95% oxygen and 5% CO2. The osmolality of the medium was established by altering the NaCl concentration and the explants were chronically maintained at different osmolalities for 24 hours (299-325mOsm/kg H20, 8-14 explants/group; 2 separate experiments). The next morning the explants were moved to fresh medium (at the same osmolality), allowed to stabilize for 2 hours, and basal VP release per hour for each explant was measured. All explants were then exposed to an acute hyperosmotic pulse of 15mOsm/Kg H2O, achieved by the addition of NaCl .The highest basal release of VP was observed in the explants maintained under isosmotic conditions (299-300 mosmoles) and was 614±110pg/hr in Experiment 1. and 330±40 pg/hr in the second experiment. These explants significantly increased VP release in response to the acute increase osmolality. Maintenance of HNS explants under chronic hypertonic conditions (above 304 mosm/kg H20) decreased basal VP release, and prevented the response to an acute hypertonic pulse.

These data indicate that chronic exposure of HNS explants to hypertonic culture medium decreases basal and osmotically stimulated VP release. This may reflect depletion of VP stores in the neural lobe, and may be comparable to the effects of chronic water deprivation or salt loading in vivo.

Supported by NIH-R01-DK-19761.

428.14

EVIDENCE FOR INVOLVEMENT OF EXCITATORY AMINO ACIDS IN OSMOTIC STIMULATION OF VASOPRESSIN RELEASE. C.D. Sladek, M. Gallagher, and C. Yagil. Departments of Neurology and Neurobiology, University of Rochester School of Medicine, Rochester, NY, 14642.

Osmotic regulation of VP release is abnormal in rats following electrolytic lesions of the region anterior and ventral to the anterior third ventricle. This suggests that osmoreceptors are located in this region, but the neurotransmitter utilized by these neurons has not been identified. In the present experiments, we examined the possibility that excitatory amino acids (EAA) are involved in this pathway by examining the effect of kynurenic acid (KA), a specific antagonist of EAA receptors, on the VP response to a 15 mOsm/kg H_2O increase in osmolality occurring over 2 hrs achieved by increasing the NaCl concentration of the culture medium. In previous experiments, this stimulus caused a significant and sustained increase in VP release. Upon returning the osmolality to baseline, VP release is inhibited, but this is followed by a paradoxical increase in VP release.

The addition of 2mM KA did not significantly alter basal VP release (39.6±5.5 pg/10 minutes, n=11) from perifused HNS explants maintained at 288 mOsm/kg H_2O. In the presence of KA, VP release was not significantly increased in response to a 15 mOsm/kg H_2O increase in osmolality occurring over 2 hours, but inhibition of VP release was observed when osmolality was returned to 288 mOsm/kg H_2O ($p=0.05$, n=5). The paradoxical increase in VP release previously observed following a decrease in osmolality did not occur. KA did not prevent stimulation of VP release in response to increasing the KCl concentration of the perifusion medium to 56mM ($p<0.01$). These observations suggest that EAAs are involved in the stimulation of VP release by increases in osmolality and also participate in the paradoxical increase in VP release observed following a decrease in osmolality. Supported by NIH RO1-DK-19761.

428.15

NUCLEUS TRACTUS SOLITARIUS (NTS) AND AREA POSTREMA (AP) PROJECT TO TUBEROMAMMILLARY NUCLEUS (TM) G.H. Beagley, M.L. Weiss, & G.I. Hatton. Psych. & Neurosci. Michigan State Univ. E. Lansing, MI 48824-1117.

Histamine-containing cell bodies of the brain have been shown to be in the TM area of the posterior hypothalamus. Projections from the histaminergic cells have been found to go to other hypothalamic nuclei, the limbic system, cortex, and subfornical organ, but few studies have investigated afferent projections to the TM. We injected the retrograde tracers, Fluoro-Gold or rhodamine labeled latex spheres, into the TM of 40 female rats to determine TM afferents. Analysis was based on the several most confined injections and the overlapping pattern of labeling. Previously reported projections were confirmed and new projections from the AP and NTS to the TM were found. Anterograde tracing studies are being done to confirm the AP-TM projection. This is evidence for chemosensory and cardiovascular input to the histaminergic cell group. Supported by NS 09140.

428.16

INHIBITION OF RAT SUPRAOPTIC NUCLEUS (SON) NEURONS BY HISTAMINE (HA) H_2-RECEPTOR ACTIVATION. Q. Z. Yang and G. I. Hatton. Neurosci. Prog. Michigan State Univ. E. Lansing, MI 48824-1117.

Histaminergic neurons of the posterior hypothalamus project to the SON and excite vasopressin cells via H_1-receptors. One immunocytochemically confirmed and two putative oxytocin neurons were reported (Soc. Neurosci. Abstr. 1988, 14:215) to be hyperpolarized by HA. We investigated intracellularly recorded responses of 24 SON neurons in hypothalamic slices to either bath or nanodrop application of HA, H_1, and H_2, agonists and antagonists. All putative or confirmed oxytocin cells were inhibited (decreased firing) and/or hyperpolarized (by 5-20 mV) by HA or the H_2-agonist dimaprit. Cimetidine (H_2-antagonist) either completely or partially reversed these effects. No effects on these cells were seen with H_1-agonists or antagonists. In one silent cell, a nanodrop of 10^{-3}M dimaprit produced a 20 mV hyperpolarization that was reversed by a similar drop of cimetidine. This cell was found to be vasopressinergic. Along with previous work, these results suggest that histaminergic input to SON differentially activates vasopressin and inhibits oxytocin neurons via H_1- and H_2-receptors, respectively. Supported by NS 16942.

428.17

RESPONSES OF PLASMA ATRIAL NATRIURETIC PEPTIDE AND ARGININE VASOPRESSIN TO OSMOTIC AND VOLUME STIMULATION. K. T. Kalogeras*, M. A. Demitrack*, L. G. Granger*, E. L. Papaioannou*, M. J. Hart*, G. P. Chrousos, and P.W. Gold* (SPON: D. LeRoith). Clinical Neuroendocrinology Branch, NIMH, and DEB, NICHD, NIH, Bethesda, MD 20892.

Arginine vasopressin (AVP) and atrial natriuretic peptide (ANP) exert both similar and dissimilar effects on salt and water metabolism. In studies of their interactions, it has been shown that ANP inhibits vasopressin actions as well as secretion, while AVP stimulates ANP release. These data suggest that ANP-AVP interactions may work preferentially to facilitate protection from hypervolemia. To further explore this question, we report here a study of the effects of 3% NaCL (given at a rate of 0.1 ml/kg/min for 2.5 hrs) on simultaneous plasma ANP and AVP secretion. ANP, AVP and Na concentrations, were measured at -30, -20, -10, and 0 min before infusion and every fifteen minutes, for 2.5 hours, thereafter. Eleven healthy males and six healthy females (studied during both the early follicular and late luteal phases) were included in the study. Hypertonic saline infusion induced a 6-fold increase in plasma AVP secretion from a basal value of 1.2±0.3 pg/ml to a peak value 7.2±1.2 pg/ml, with a parallel 3-fold increase in plasma ANP from 49.9±7.7 to 146.6±19.7 pg/ml. Both the rise in plasma AVP and ANP correlated significantly with the rise in plasma sodium, with a mean (±SEM) correlation coefficient of 0.90±0.02 for AVP and 0.88 ±0.02 for ANP ($p<0.001$ for both). No significant differences were observed in the correlation coefficient, the slope and the osmotic threshold, between male and female subjects and between follicular and luteal phase of the menstrual cycle. The rise in plasma ANP correlated significantly with the rise in plasma AVP with a mean correlation coefficient of 0.84±0.03 ($p<0.001$) and a slope or sensitivity of 20.71±2.75 pg/ml ANP per pg/ml AVP. The positive correlation between plasma AVP and ANP suggest that ANP and AVP work together to correct hyperosmolality.

428.18

ISOPROTERENOL-INDUCED MYOCARDIAL INFARCTION IN THE RAT: EXPERIMENTAL EVIDENCE. MJ Krieman* and WH Vogel, Department of Pharmacology, Thomas Jefferson University, Philadelphia, PA.

As a prelude to studying the stress response of rats with cardiac damage, we needed to establish in our lab the animal model for isoproterenol(ISO)-induced myocardial infarction. Male Sprague-Dawley rats between 350-450g had jugular catheters and bipolar EKG leads implanted. 48 hours later, baseline blood samples and EKG traces were taken. Rats then received a 300mg/kg ISO, 500mg/kg ISO, or .9% saline injection s.c. Blood samples and EKG traces were taken for 48 hours. The control group remained unchanged. The ISO-treated groups showed changes in all parameters. Total serum CPK activity increased acutely, peaking at three hours. Qualitative analysis of the isoenzymes showed CPK-MB bands apparent from 3-12 hours. Common EKG deviations were the appearance of S-waves and flattening or inversion of t-waves. Histological findings included widespread necrosis characterized by leucocyte infiltration and destruction of myofibril cells, especially in the left ventricle. Thus, our data provides comprehensive evidence for isoproterenol-induced myocardial infarction in the rat. Subsequent studies will focus on the sympathetic response to stress of these cardiac-damaged animals as compared to normal, healthy animals.

428.19

LAMINA TERMINALIS INPUT TO RAT SUPRAOPTIC NUCLEUS VISUALIZED WITH RETROGRADE TRANSPORT OF LABELED MICROSPHERES. J. T. Cunningham, R. Nissen, and L. P. Renaud. Center for Research in Neuroscience, Montreal General Hospital & McGill University, Montreal, Canada, H3G 1A4

The lamina terminalis region is a major input to the supraoptic nucleus (SON). To define these projections more precisely, we evaluated retrograde transport of rhodamine-labeled (Rh) or flourescent green-congigated (Gr) latex beads injected into each SON using a transpharyngeal approach. Animals were perfused 8-22 hrs later. Single SON injections yielded 15-40 labeled cells in the organum vasculosum of the lamina terminalis (OVLT), 130-200 in the median preoptic nucleus (MnPO), and 200-250 in the subfornical organ (SFO). Cells in the MnPO and SFO were widely distributed while the projection from OVLT was primarily ipsilateral. Bilateral SON injections using Rh and Gr beads double labeled up to 4% of retrogradely filled cells in the OVLT, 1% in the MnPO, and 5% in SFO. Bilateral projections from these midline structures represent a minority of their input to the SON. (Supported by MRC and NIMH fellowship MH09766-01X1 BPN-2)

429.1

A 16 KD NUCLEAR PHOSPHOPROTEIN MAY BE INVOLVED IN POMC GENE REGULATION. N. Margolis and T. Reisine. Dept. of Pharmacology, Univ. of Pennsylvania, Phila., PA 19104

Control of proopiomelanocortin (POMC) gene expression is an important event in the body's response to stress. Corticotropin releasing factor (CRF) and forskolin, which increase cellular cAMP levels, stimulate POMC gene transcription in the anterior pituitary by activating cAMP-dependent protein kinase. We are attempting to identify nuclear substrates of the kinase which might play a role in POMC gene regulation.

Using AtT-20 cells,a mouse anterior pituitary corticotroph tumor cell line, as a model for POMC gene regulation, we have identified several nuclear proteins which are phosphorylated in response to CRF and forskolin. Among these proteins is a 16 kd protein which appears to be tightly bound to a 9M urea-insoluble fraction of the nuclei and is only released from this fraction when it is sonicated. The phosphoprotein can be visualized on an SDS-PAGE gel by Coomasie blue staining, indicating its abundance in AtT-20 cell nuclei. Preliminary evidence indicates that the phosphorylated protein binds to DNA-cellulose, suggesting that this 16 kd protein might interact with DNA in the cell. The phosphoprotein has been partially purified by elution from SDS-PAGE gels and is currently being characterized using an *in vitro* phosphorylation assay. Future work will include examination of the ability of the phosphoprotein to bind to the 5' promoter region of the POMC gene. Supported by NIH grant DK37407 and an Americian Heart Association grant-in aid.

429.2

CALCIUM-DEPENDENT PROTEIN PHOSPHORYLATION IN THE RODENT ANTERIOR PITUITARY. J.C. Pryor, S.T. Cain, C.B. Nemeroff. Depts. of Psychiat. & Pharmacol., Duke Univ. Med. Ctr., Durham, NC 27710.

Calcium mobilization has been implicated in the sequence of steps that leads to the stimulated secretion of anterior pituitary hormones. Changes in the phosphorylation state of individual protein substrates is regarded as one of the predominant mechanisms by which calcium ions modulate hormone release. Previous reports have documented that calcium/phospholipid- and calcium/calmodulin-dependent protein kinases stimulate protein phosphorylation in both sheep and bovine anterior pituitary preparations. We have extended these investigations to evaluate the presence of calcium-dependent phosphoprotein substrates in the rodent anterior pituitary.

Adult, male rats were decapitated, and the anterior pituitaries were harvested and homogenized. Aliquots were phosphorylated in the presence of 10 μM ATP (final concentration) containing [γ^{32}P-ATP], along with either vehicle, calcium (10 μM,), calmodulin (0.05 μg/ml), phosphatidylserine (300 μg/ml), calcium/calmodulin, calcium/ phosphatidylserine. The reaction was carried out for 1 min. The phosphoproteins were separated on SDS-polyacrylamide gels and autoradiographs prepared. Densitometric analysis of the autoradiographs indicate the preferential phosphorylation of 54 and 74 KD MW anterior pituitary proteins by calcium alone. Proteins of 66, 32, and 17 KD MW were preferentially phosphorylated in the presence of calmodulin or phosphatidylserine. Calmodulin stimulated the phosphorylation of a 19 KD MW protein. (Supported by NIMH MH-42088 and the N.C. United Way Fund.)

429.3

CATECHOLAMINES AND THE ACTH RESPONSE TO NICOTINE. S.G. Matta*, K.M. McAllen*, and B. M. Sharp*. (SPON: F. Wilson) Minneapolis Med. Res. Fndn. and Depts of Medicine, Hennepin County Medical Cntr. and Univ. of Minnesota, Minneapolis, MN 55404.

Nicotine(N)-stimulated ACTH release depends upon the activation of central mechanisms, particularily in areas accessible to N from the fourth (IV) ventricle. This region contains catecholaminergic (CAT) nuclei and N stimulates the release of CATs. Moreover, CATs induce ACTH secretion via the release of hypothalamic CRF. Thus, the current studies determined whether central CATs are involved in N-stimulated ACTH secretion. 6-hydroxy-dopamine (6-OH-DA) or vehicle (SHAM) were injected into the lateral ventricle of rats; after 9 d, rats were given saline or 0.03 or 0.05 mg/kg bwt N i.v. ACTH values (pg/ml) are mean$\pm$sem:

	0min	3min	7min	15min	30min
Saline: SHAM	24$\pm$6	27$\pm$2	23$\pm$4	36$\pm$7	66$\pm$24
6-OH-DA	32$\pm$9	42$\pm$11	34$\pm$7	37$\pm$7	44$\pm$10
0.03 N: SHAM	17$\pm$6	199$\pm$97	264$\pm$114	183$\pm$51	113$\pm$26
6-OH-DA	11$\pm$5	53$\pm$12	57$\pm$4	46$\pm$5	53$\pm$8
0.05 N: SHAM	29$\pm$5	290$\pm$85	426$\pm$154	352$\pm$178	167$\pm$99
6-OH-DA	32$\pm$7	127$\pm$39	84$\pm$25	61$\pm$18	48$\pm$35

In addition, the ACTH response to either stress was reduced from 626$\pm$93 (SHAM) to 327$\pm$78 (6-OH-DA) ($p<0.05$). This indicates that CATs may be involved as mediators of the ACTH response to nicotine. (Supported by DA03977).

429.4

MONOAMINE MEDIATION OF COCAINE-INDUCED HPA ACTIVATION. **B.Borowsky and C.M.Kuhn. Department of Pharmacology, Duke University Medical Center, Durham, North Carolina, 27710.**

The acute administration of cocaine (5-20 mg/kg,ip) to rats produced a dose-dependent elevation in both serum corticosterone (CS) and plasma adrenocorticotropin hormone (ACTH). These rises were maximal at 30 min and returned to basal values by 60 min. More selective DA uptake blockers GBR12909 and nomifensine also stimulated hypothalamo-pituitary-adrenal (HPA) axis activity, while the local anesthetic, procaine, did not. Pretreatment with haloperidol (0.2 mg/kg) significantly attenuated the increases in CS and ACTH elicited by cocaine, as well as the elevation in ACTH produced by GBR12909. Pretreatment with the D1 antagonist, SCH23390, or the 5HT2 antagonist, ketanserin, but not the alpha-1 antagonist, prazosin, significantly decreased the ACTH rises following cocaine. Intracerebroventricular 6-OHDA lesions also significantly attenuated the ACTH response to cocaine. Following the repeated administration of cocaine (15 mg/kg, twice daily, 7 days), rats displayed a sensitized behavioral response to cocaine challenge while the HPA response to cocaine or saline was unaltered. The present results support a stimulatory role for dopamine, involving both D1 and D2 receptor subtypes, in regulation of HPA activity. However, both DA and 5HT could contribute to the adrenocortical stimulation by cocaine.

429.5

EFFECTS OF THE OPIATE-ANTAGONIST NALTREXONE ON STRESS-INDUCED PEPTIDE RELEASE FROM THE RAT INTERMEDIATE PITUITARY. **J.A. Carr, L.C. Saland, A. Samora*, E. Vigil-Palmer* and S. Desai*. Dept. of Anatomy, Univ. of New Mexico Sch. Med., Albuquerque, NM 87131.**

We have previously reported that acute restraint stress results in ultrastructural evidence for enhanced release of pro-opiomelanocortin (POMC)-derived peptides from the intermediate lobe (IL) of the rat pituitary (Carr et al., 1989, Anat. Rec. 223:22A). In this study, we investigated the possibility that endogenous opioid peptides mediate peptide release from the IL during stress. Male Sprague-Dawley rats were injected ip with saline or one of two doses of the opiate-antagonist naltrexone (1 mg/kg or 10 mg/kg) 30 min prior to being placed in plexiglass restraint cages. Unstressed rats received similar injections. Pituitaries were fixed in 2.5% glutaraldehyde-1% paraformaldehyde in 0.1 M phosphate buffer for subsequent TEM. IL cells in unstressed animals contained numerous membrane-bound granules of various electron densities. As expected, there was a significant decrease in the granular content of IL cells in saline-treated rats stressed for 30 min. Pretreatment of rats with either 1 or 10 mg/kg naltrexone did not attenuate the degranulation of IL cells during stress. Conversely, naltrexone treatment appeared to potentiate the degranulation of IL cells during stress. These results indicate that stress-induced release of POMC peptides from the IL is not mediated by opioid receptors. Supported by NIH NS21256 and RR08139 (LCS).

429.6

INTERACTIONS OF CORTICOTROPIN-RELEASING FACTOR (CRF) AND α-HELICAL CRF ON PEPTIDE RELEASE FROM RAT NEUROINTERMEDIATE LOBES IN VITRO. **E. Vigil-Palmer*, A. Samora*, S. Desai*, J.A. Carr and L.C. Saland.(SPON: S. Rogers). Dept. of Anatomy, Univ. of New Mexico Sch. Med., Albuquerque, NM 87131.**

CRF stimulates proopiomelanocortin (POMC) peptide release from both anterior (AL) and intermediate (IL) pituitary lobes. Dopamine, an inhibitor of POMC release from the IL, reduces CRF stimulation effects in the IL in vitro (Saland et al, 1988, Neuropeptides 12: 59). The synthetic antagonist, alpha-helical CRF (α-Hel), suppresses CRF effects in AL POMC cells. Here, we demonstrate inhibition of POMC secretion from the IL by α-Hel. Neurointermediate lobes (NILS) from ether anesthetized adult male Sprague-Dawley rats were incubated in Gibco medium including glucose, glutamine and 0.1 mM bacitracin, for 90 minutes. CRF (10^{-9} M) was added, with or without α-Hel (10^{-7} M). NILS were also incubated with CRF followed by addition of α-Hel at 30 minutes, or the converse. Aliquots of media were assayed for β-endorphin (END) immunoreactivity by radioimmunoassay. Alpha-Hel reduced β-END release in all cases, whether added with CRF, or before or after CRF. Immune staining for POMC peptides of tissue taken at 90 minutes confirmed inhibition of peptide release after α-Hel treatment. Electron microscopy of tissue fixed at 90 minutes showed cytologic changes consistent with modulation of granule secretion by α-Hel. POMC cells of the rat IL are thus responsive to a specific inhibitor of CRF to suppress peptide release. Supported by NIH NS 21256 and RR-08139 (LCS).

429.7

HYPOTHALAMIC AND PITUITARY METABOLISM ACTIVATED BY ELECTRICAL STIMULATION OF THE AREA POSTREMA. S.W. Shaver, D.S. Wainman*, K.M. Wall*, A.V. Ferguson and P.M. Gross. Depts. Surg. & Physiol., Queen's Univ., Kingston, ON K7L 3N6.

Polysynaptic pathways link hypothalamic nuclei and the neurohypophysis with the area postrema (AP), a medullary nucleus involved in viscerosensory, autonomic, and endocrine regulation. Using the [^{14}C]deoxyglucose method in halothane-anesthetized rats, we tested the effect of electrical stimulation (ES; 75-150 μA, 15 Hz, 1 ms) of the AP on metabolic activity in forebrain components of this neural circuit. ES produced reflex hypotension (-20% in mean arterial blood pressure) and 22 to 63% increases in glucose metabolism of first-order AP projections in the medulla and pons. Forebrain sites displaying elevated rates of glucose metabolism were the hypothalamic paraventricular (+38%), supraoptic (+22%), and suprachiasmatic nuclei (+47%), median eminence (+29%), and pituitary neural lobe (+104%). Equivalent hypotension induced by hemorrhage in rats not receiving ES altered neither hypothalamic nor neurohypophysial metabolism, results confirming the specific effects of ES. These studies identify selective functional responses in hypothalamic nuclei and the pituitary gland mediated over long neural pathways from stimulation of a brainstem nucleus. Thus, we speculate that controls for pituitary secretions, body fluid homeostasis, and blood pressure are influenced by ascending projections from the AP.

429.8

ATRIOPEPTIN INHIBITS PITUITARY CORTICOTROPIN RELEASE IN VITRO: INVOLVEMENT OF CYCLIC GMP AND POTASSIUM CHANNELS. F.A.Antoni* and G.Dayanithi* (SPON: BRA, U.K.) MRC Brain Metabolism Unit, Univ. Dept.of Pharmacology, Edinburgh, U.K. and Dept.of Human Anatomy, Univ. Oxford, Oxford, U.K.

The physiological role of atriopeptin produced by hypothalamic neurons that project to the external layer of the median eminence is unknown. Here we report the analysis of the action of 5-28 residue atriopeptin (ANF) on corticotropin (ACTH) release by isolated rat anterior pituitary cells in a perifusion system.

The secretion of ACTH was stimulated by the combined administration of 0.05 nM corticotropin-releasing factor and 0.5 nM arginine-vasopressin (CRF/AVP). Repeated exposure to CRF/AVP for 2.5 min at 30 min intervals resulted in a gradual increase of the net ACTH response from 3-4x basal release to a plateau of 8-10x basal by 2 h. ANF (0.1 nM) completely prevented the increase of the hormonal response while 0.01 nM of the peptide attenuated it by 40%. Once the maximal ACTH response was attained, ANF still inhibited CRF/AVP induced ACTH release, albeit with a lower (cca. 10x) potency. In contrast, ANF failed to inhibit ACTH release evoked by the combined stimulus of 10 nM phorbol dibutyrate and 200 nM ionomycin.

The actions of ANF in this system were mimicked by 8-Br-cGMP, moreover, ANF increased the level of cGMP in the cells, and an ANF analogue largely devoid of guanylyl-cyclase stimulating activity was a weak inhibitor of ACTH release. Finally, blockers of K channels, such as tetraethyl ammonium (5 mM), 4-aminopyridine (5mM) and quinine (0.1 mM) all blocked the inhibitory action of ANF, but had no marked effect on CRF/AVP induced ACTH release when given alone.

In summary, ANF inhibits secretagogue-induced ACTH release by activating guanylyl cyclase, and the cGMP produced may influence the flux of K^+ and/or Ca^{2+} through the plasma membrane. We propose that ANF is a hypothalamic ACTH-release inhibiting hormone.

429.9

INCREASED POMC GENE EXPRESSION IN ANTERIOR PITUITARIES OF SUICIDE VICTIMS: AN *IN SITU* HYBRIDIIZATION STUDY. J.F. Lopez*, S.J. Watson, M. Arato*, M. Palkovitz*, S. Burke* and H. Akil (SPON: M. Akil). Mental Health Research Institute, Ann Arbor, MI 48109-0720; National Institute for Nervous and Mental Diseases, Budapest, Hungary.

Suicidal behavior has been associated with hypothalamic-pituitary-adrenal overactivity in humans, as measured by increased corticosteroid secretion (Yehuda, et al., Neurol. Clin., 6:83, 1988). To investigate if this overactivity is reflected at the pituitary level, we have studied the accumulation of pro-opiomelanocortin (POMC) mRNA, the molecule coding for the ACTH/β-endorphin precursor, in human anterior pituitaries.

Pituitaries from 7 suicide victims and 11 cardiac deaths were sectioned in to 10 μ slides, stained with thionin and processed for *in situ* hybridization using a riboprobe complementary to human POMC mRNA. To correct for possible post-mortem cell loss, hybridization with P1B15, a cDNA complementary to rat cyclophilin mRNA, was used in adjacent sections. Quantification with a computerized image analysis system (ICC/LOATS) revealed a 25% increase in POMC message in suicide victims. Analysis of the corticotrophic cell clumps showed that the suicide victims had higher POMC mRNA density per cell (p=0.04, t-test) and larger corticotrophic cell size (p=0.04) than the cardiac death victims. There were no differences in P1B15 message between the two groups. No correlation with age or post-mortem time were detected. The relationship of human glucocorticoid receptor mRNA and β-endorphin peptide content to these changes is currently under investigation in the same pituitaries and will be reported. Supported by NRSA MH09632 (J.F.L.) and NIMH MH422251 (S.W. and H.A.)

429.10

SPECIES DIFFERENCES IN PLASMA ß-ENDORPHIN/ß-LIPOTROPHIN RATIOS. E.H. Mougey, K.L. Huhman, J.L. Meyerhoff and M.A. Marrazzi. Department of Medical Neurosciences, Walter Reed Army Institute of Research, Washington, D.C. 20307-5100 and Department of Pharmacology, Wayne State University School of Medicine, Detroit, MI 48201.

Human and animal plasmas were extracted using PrepPAK-500/C_{18} and the endorphins (ß-EP and ß-LPH) were separated by sequential solvent elution. Plasma was added to 143 mg of the C_{18} adsorbent along with an equal volume of acidified water. After centrifugation of the tubes and aspiration of the supernatants the C_{18} was washed three times with water. ß-LPH was eluted with two 1 ml rinses of acetone:water and ß-EP was eluted with two 1 ml rinses of acetone:water:TFA. The extracts were evaporated to dryness under nitrogen gas at 45°C and the residues were taken up in RIA buffer and assayed using a commercial kit (NEN) for human samples and an in-house RIA for the animal samples. Both antisera cross-react with ß-LPH.

Male human subjects (N=10) had a mean ß-EP level of 7.2±2.4 pg/ml and a mean ß-LPH level of 27.7±5.9 pg/ml (expressed as ß-EP equivalents) for a mean plasma ß-EP/ß-LPH ratio of 0.263±0.078. Male Sprague Dawley rats (N=12, 90 days old) had a mean ß-EP level of 355±42 pg/ml and a mean ß-LPH level of 266±96 pg/ml, ratio=1.46±0.43. Female mice (N=8, CBA/J, Jackson Labs.) had a mean ß-EP level of 30.6±18.0 and a mean ß-LPH level of 293±39 pg/ml, ratio=0.104±0.058. Male Syrian hamsters (N=12) had a mean ß-EP level of 246±49 pg/ml and a mean ß-LPH level of 98±29 pg/ml for a ratio of 2.65±0.64.

429.11

HYPOTHALAMIC-PITUITARY-ADRENAL DYSFUNCTION IS ASSOCIATED WITH COGNITIVE IMPAIRMENTS IN AGED RATS. A. Issa, S. Gauthier, W. Rowe, & M.J. Meaney. Douglas Hospital Research Ctr., Depts. of Psychiatry and Neurology and Neurosurgery, McGill Univ., Montreal H4H 1R3, Canada.

Increased activity of the hypothalamic-pituitary-adrenal (HPA) axis is associated with age-related neuropathology. The hippocampus, a critical brain region in learning and memory, is especially sensitive to glucocorticoids (GCs), and chronic exposure to elevated GCs results in the loss of hippocampal neurons (Sapolsky et. al, J. Neurosci., 1985).

To assess the relationship between HPA activity and cognitive dysfunction in later life, we screened over 100 aged (24-29 month old) rats using the Morris swim maze. Three groups were then compared: aged cognitively-impaired, aged-cognitively unimpaired, and young adult (6-8 months of age). Plasma levels of ACTH and corticosterone (B) were significantly elevated during the PM phase of the cycle in the aged-impaired animals. Moreover, the aged-impaired animals hypersecreted B following the termination of a stressor. HPA activity under both basal and stressful conditions was similar in the aged-unimpaired and young adult rats. Type II, glucocorticoid receptor binding in hippocampus, measured using [3H]RU 28362, was lower in both groups of aged animals, however, the receptor loss was significantly greater in the aged-impaired animals.

These data suggest that HPA dysfunction in the rat is associated with neuropathology and does not occur merely as a function of age. Taken together with our previous studies (see Meaney et. al, Science, 1988) these findings also suggest that individual differences in HPA function in later life are a major predictor of neuropathology in later life.

429.12

BASAL HYPOTHALAMIC-PITUITARY-ADRENAL FUNCTION IN AGED HANDLED AND NONHANDLED RATS. V. Viau, S. Sharma, D.H. Aitken, & M.J. Meaney. Douglas Hospital Research Ctr., Dept. Psychiatry, McGill Univ., Montreal H4H 1R3, Canada.

Adult rats handled (H) for the first 3 weeks of life show increased negative-feedback sensitivity to circulating glucocorticoids, an effect that endures throughout the life of the animal (Meaney et al., Science, 1988; Neuroendocrinology, In press). With age, basal corticosterone (B) levels rise dramatically during the PM phase of the cycle in nonhandled (NH), but not in H rats. There is a moderate increase in basal plasma B during the AM phase with age in both H and NH rats. Plasma levels of corticoid-binding globulin (CBG) are similar in young H, young NH animals, and old H animals. During the PM phase of the cycle CBG levels in the old NH animals are significantly ($P<0.01$) reduced and free levels of B are 3-4 fold higher than in old H animals.

Type II, glucocorticoid receptor binding in hippocampus, measured using [3H]RU 28362, was lower in both groups of aged animals, however, the receptor loss was significantly ($P<0.05$) greater in the aged NH animals. Type I, glucocorticoid receptor binding in hippocampus, measured using [3H]B in the presence of cold RU 28362, was lower in both aged H and NH animals. These data indicate that hypersecretion of B in the aged NH animals is substantial, especially in the PM phase of the cycle. Differences in PM, basal B secretion appear to be associated with changes in type II receptors, whereas the moderate, aged-related increase in AM, basal B in both H and NH animals may be associated with the loss of type I receptors.

429.13

NEUROENDOCRINE RESPONSES TO m-CHLOROPHENYLPIPERAZINE (m-CPP), A RELATIVELY SELECTIVE $5HT_{1B}$ AGONIST. J. Saydoff, P.A. Rittenhouse, M. Carnes, M.S. Brownfield, and L.D. Van de Kar. Sch. Vet. Med., Univ. of Wisconsin, Madison, WI 53706, Sch. Med., Loyola Univ., Maywood, IL 60153, and Veterans Admin. Hosp. Madison, WI 53705.

To determine the potency of m-CPP in stimulating neuroendocrine function we compared the secretion of corticosterone, renin, and vasopressin in the rat. Groups of eight rats were administered m-CPP, 0-20 mg/kg, i.p. Thirty minutes later rats were killed and trunk blood was collected and processed for radioimmunoassay of plasma corticosterone, renin, and vasopressin. Corticosterone secretion was stimulated by m-CPP in a dose-response manner with a minimum effective dose of 3 mg/kg and remained elevated at all higher doses. In contrast, the renin dose-response curve was shifted to the right with a minumum effective dose of 10 mg/kg. Vasopressin secretion was unresponsive to m-CPP at any dose tested.

Comparison of the different potencies of m-CPP revealed in the dose-response curves for these hormones may suggest that different receptor mechanisms are involved. This conclusion would be consistent with previous studies that have previously shown that corticosterone secretion is stimulated by a $5HT_{1A}$ mechanism while renin and vasopressin are stimulated by a $5HT_2$ mechanism. It should be mentioned that the minimum effective dose of m-CPP needed to elict corticosterone secretion was about five to ten times higher than that required by $5HT_{1A}$ agonists, suggesting that m-CPP might have some $5HT_{1A}$ activity. Renin secretion is also stimulated by RU24969, a drug that is structurally similar to m-CPP, but this effect was blocked by ritanserin, a $5HT_2$ antagonist. This might imply that m-CPP has some $5HT_2$ agonistic properties.

The results show that m-CPP stimulates the secetion of corticosterone and renin, but not vasopressin. The specific receptors involved and their sites that mediate these neuroendocrine responses remain to be established.

429.14

ADRENOCORTICOTROPIN HYPERSECRETION ASSOCIATED WITH DIABETES MELLITUS RESULTS FROM AN INCREASED RELEASE OF HYPOTHALAMIC CORTICOTROPIN RELEASING FACTOR. C. A. Johnston and C. Chapman*. College of Pharmacy, Wash. State Univ., Pullman, WA 99164-6510.

Adrenal hypertrophy, and increased plasma levels of adrenocorticotropin (ACTH) and corticosterone have been observed in diabetics both experimentally and clinically. The central mechanisms responsible for this diabetes-associated hypersecretion of ACTH are not known. The present study examined the effects of experimentally-induced diabetes mellitus [streptozotocin (STZ), 65 mg/kg BW, iv] on the concentrations, release and anterior pituitary (AP) responsiveness to two major physiologically relevant central ACTH secretagogues, corticotropin releasing factor (CRF) and arginine vasopressin (AVP) in male Sprague-Dawley rats. Diabetes was confirmed by monitoring daily water intake and measuring blood glucose levels. Content of CRF and AVP were measured in acid extracts of medial basal hypothalamic (MBH) tissues dissected from diabetic or control animals using specific RIAs. Basal and potassium-induced release of CRF and AVP was evaluated in vitro from MBH tissues. The in vivo ACTH response to an iv injection of either CRF (1 ug/rat) or AVP (1 nmole/rat) was assessed using chronic indwelling jugular cannulae in unanesthetized, freely moving rats. The data indicate that the diabetes-associated hypersecretion of ACTH is not due to alterations in the content/release of central AVP or the ACTH response of the AP to CRF or AVP, but appears to be caused by an enhanced release of CRF from the hypothalamus. This work was supported by a grant from the Diabetes Research and Education Foundation.

429.15

LIPOCORTINS AND ASTROGLIAL PROSTANOID SYNTHESIS P.J.Gebicke-Haerter*, A.Schobert*, P.Dieter*, G. Hertting*, (SPON: J. Geer)Inst. of Pharmacol.& Biochem., Univ. Freiburg, 78 Freiburg, F.R.G.

Increased synthesis of lipocortins (LC) is believed an important part of the anti-inflammatory effects of glucocorticoids. WESTERN blot analyses of LC using specific antisera against LC I(resp. its 32 kDa fragment) and against LC II (Pepinsky et al., J.Biol.Chem.261,4239) revealed that : 1. LC I and II levels were very low in rat brain and liver, 2. LC I was specifically induced in cultured astrocytes, 3. LC I-32 kDa (or LC III, cross-reacting with antisera) was more prominent in microglia and was the predominant form in Kupffer cells. Hence, regulation of LC I-III synthesis is cell type specific.

4. LC I immunoprecipitated from ^{35}S-methionine-labeled astrocytes was increased upon dexamethasone treatment, 5. Glucocorticoids inhibited Ca^{2+}-ionophore A_{23187}-, TPA-, or ATP (Gebicke-Haerter et al., N.S.Arch.Pharm.338,704)-stimulated prostaglandin D_2 formation to various extent dependent from type of stimulus. 6. A 170 kDa lipocortin-like protein was found to cross-react with LC I antisera. Cultured astrocytes appear to be a good model system to study the regulation of phospholipase A_2 activity by LC I.

429.16

EFFECT OF HYPO- AND HYPERTHYROIDISM ON HYPOTHALAMIC-PITUITARY-ADRENAL AXIS RESPONSIVENESS TO NEUROPEPTIDE Y (NPY). E.O. Johnson*, T.C. Kamilaris*, A.E. Calogero,* A. Spalter*, P.W. Gold, and G.P. Chrousos. DEB/NICHD&CNB/NIMH, Bethesda, MD,

Hypothyroidism is associated with decreased and hyperthyroidism with increased activity of both the hypothalamic-pituitary-adrenal (HPA) axis and the sympathetic system. Neuropeptide Y (NPY), a 36 amino-acid peptide, is co-secreted with catecholamines during stress. We have shown that NPY activates HPA axis at both the hypothalamus and the adrenal cortex by causing CRH and corticosterone release, respectively. To examine the hypothesis that changes in thyroid status might affect the interactions between NPY and the HPA axis, we studied chronically cannulated, freely moving male rats with long-standing (60 days) hypothyroidism, hyperthyroidism, or euthyroidism. In all animals, we measured plasma concentrations of ACTH and corticosterone before and after an iv bolus of 10µg/100g BW NPY. Hypothyroid rats demonstrated significantly lower plasma ACTH and corticosterone concentrations after NPY administration than those of control rats. In contrast, no alteration in the basal and NPY-stimulated plasma ACTH was observed in the hyperthyroid animals. However, these animals had consistently elevated plasma corticosterone concentrations, confirming prior studies demonstrating hypercorticosteronism in hyperthyroid rats. We conclude that hypothyroidism is associated with decreases in NPY-induced HPA activation. The relative importance between this neuropeptide and the co-secreted catecholamines in regulating CRH secretion remains to be seen.

429.17

ADRENAL AND AUTONOMIC RESPONSES TO NOXIOUS STIMULI IN THE CAT: INTERACTION WITH BLOOD LOSS. D.A.Bereiter, A.P.Benetti* and K.V.Thrivikraman. Depts. Neurobiol. & Surgery, Brown Univ./R.I.Hospital, Providence, RI 02903.

The neuroendocrine responses to injury are mediated in part by nociceptive and cardiovascular afferent signals; however, the underlying central neural mechanisms are not well defined. Previously, we reported that glutamate activation of neurons within trigeminal subnucleus caudalis evoked adrenal and autonomic responses consistent with those seen after injury. Presently, the adrenal venous secretion of epinephrine (E) and plasma ACTH were assessed after noxious trigeminal stimuli (corneal heat, 52°C), after moderate blood loss (10ml/kg), and after pairing of both stimuli in chloralose-anesthetized cats. Also, the neurochemical input to the caudal raphe nuclei (Rpa) was examined by a push-pull perfusion technique to determine if monoaminergic input to this CNS region reflected the observed endocrine or autonomic responses. Perfusate was sampled (25µl art. CSF/min over 5 min) and processed by HPLC-EC. Corneal heat evoked an increase in arterial pressure (MAP) and in E (+7±2 ng/min), whereas plasma ACTH was not affected (-7±15 pg/ml). In contrast, blood loss evoked a decrease in MAP, but an increase in E (+14±3 ng/min) and in ACTH (+137±88 pg/ml). Presentation of corneal heat during blood loss evoked an exaggerated ACTH response (+233±97 pg/ml, P<.01), whereas the increase in E (+12±7 ng/min) was similar to that after blood loss alone. Noradrenergic input to the Rpa increased after corneal heat, but not after blood loss. Summary: corneal afferent nerve activity reliably increased the adrenal secretion of catecholamines (CA) and arterial pressure, but corneal heat alone did not affect ACTH. The potentiated release of ACTH by corneal heat during blood loss indicated an interaction between trigeminal nociceptive and cardiovascular afferents in the control of ACTH, whereas the adrenal secretion of CA was not affected by such an interaction. The perfusate results were consistent with a role for Rpa in the integration of nociceptive input and in control of cardiovascular function, but did not correlate well with the secretion of CA or with the interaction of stimuli that augmented plasma ACTH. Supported by NIH grant NS26137.

429.18

EFFECTS OF V1 VERSUS V1+V2 VASOPRESSIN ANTAGONISTS ON THE ACTH AND GLUCOCORTICOID RESPONSES TO HYPOTENSION IN CONSCIOUS DOGS. V.L. Brooks* (SPON: R. Boyle). Dept. of Physiology, Oregon Hlth Sci Univ., Portland, OR 97201.

Pretreatment of conscious dogs with the V1 vasopressin (VP) antagonist (V1-A), $d(CH_2)_5Tyr(Me)AVP$, attenuates the increases in plasma ACTH and glucocorticoid (GC) concentrations produced by hypotension. We investigated whether an attenuation of the ACTH and GC responses would also be found in dogs pretreated with the combined V1+V2 VP antagonist (V1V2-A), $desGly^9d(CH_2)_5[D\text{-}Tyr(Et^2)]VAVP$. In untreated dogs (n=14), four consecutive, 15 min IV infusions of the vasodilator nitroprusside (NP), at increasing doses of 0.3, 0.6, 1.5 and 3.0 $\mu g \cdot kg^{-1} \cdot min^{-1}$, gradually decreased mean arterial pressure from 103 ± 3 to 86 ± 3 mmHg (P<0.001), increased GC from 16 ± 2 to 71 ± 6 ng/ml (P<0.001) and increased ACTH from 31 ± 3 to 139 ± 25 pg/ml (P<0.001). Both V1-A (6 ± 1 µg/kg; n=7) and V1V2-A (100 µg/kg; n=7) reduced the CC and ACTH responses to hypotension; however, there was no difference in the effects of V1-A versus V1V2-A. In addition, the increases in GC associated with given increases in ACTH were less in dogs pretreated with either VP antagonist. These data indicate that endogenous VP is required for normal ACTH and GC responses to hypotension, and that this effect can be blocked by either a V1 or a combined V1+V2 antagonist. Furthermore, VP may increase GC during hypotension in part by an effect independent of the regulation of ACTH secretion. Supported by NIH Grant HL 35872.

429.19

IN SITU HYBRIDIZATION OF VASOPRESSIN mRNA IN PARVOCELLULAR PARAVENTRICULAR (PVN) NEURONS IS ABOLISHED IN ADRENALECTOMIZED (ADX) RATS BY LOW CORTICOSTERONE.

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ADX stimulates increases in CRF and vasopressin(AVP) secretion and expression in parvocellular PVN, resulting in marked stimulation of ACTH secretion. Corticosterone (B) replacement of ADX rats restores ACTH to normal. To determine whether inhibition by B of ADX-associated increases in AVP expression in parvocellular PVN is consistent with inhibition mediated by high affinity type I (K_D 0.5 nM) or lower affinity type II (K_D 2.5-5nM) B receptors, ADX rats were replaced with 0, 25 or 75% B pellets sc, and killed in the AM on d5. Blood and PVN brain sections were collected from these rats and sham controls; sections were hybridized with an ^{35}S-labeled 35-base oligomer for AVP, emulsion-dipped, developed and examined for location of silver grains. Grains over parvocellular cells in PVN were observed in ADX, but were restricted to magnocellular cells in Shams and both B-treated ADX groups. Plasma ACTH was normalized in the 25% B groups at plasma B concentrations of 80nM total, or 1 nM estimated free B (Endo. 121:1104, 1987). We conclude that free B concentrations lower than the K_D for type II, but higher than the K_D for type I receptors are sufficient to normalize ADX-induced increases in AVP mRNA in parvocellular neurons of the PVN, suggesting a type-I mediated effect.

HYPOTHALAMIC-PITUITARY-GONADAL REGULATION II

430.1

DENSITY OF SYNAPTIC INPUT IS IDENTICAL TO SMOOTH-CONTOURED AND THORNY GnRH NEURONS IN THE MALE RAT.

J.W. Witkin and K.A. Demasio*. Dept. Anatomy and Cell Biology, Columbia Univ. Coll. P&S, New York, NY 10032

GnRH neurons are typically smooth-contoured and fusiform in shape with a process extending at each pole. In the rat and other species, there is a subpopulation of these neurons which has an irregular contour with spiny projections from the cell soma and proximal processes. It is not known whether this difference is functionally significant, but the relative numbers of thorny GnRH neurons is reduced in immature (Wray and Hoffman, Neuroend.43:93,'86) and castrate rats (Witkin, Neuroend., in press). In order to discern whether the thorny neurons are more densely innervated (as has been suggested, Jennes et al., J. Comp. Neurol.,232:534,'85), 4 adult male rats were deeply anesthetized with pentobarbital and perfused with paraformaldehyde/glutaraldehyde and brain sections from the preoptic area were treated immunohistochemically for the ultrastructural demonstration of GnRH, using the LR1 antibody (Benoit). 5 smooth and 5 thorny neurons were identified in 1 um sections and a series of ultrathin sections at 3 depths prepared. From photomicrographs of these cells, the percent of plasma membrane in synaptic contact was determined, and found to be the same in the two groups of neurons.

To ascertain whether these cells might differ in some other aspects, the relative representations of subcellular organelles in the two shapes were compared using point counting stereology. Smooth contoured neurons had more and larger nucleoli but a smaller volume fraction of Golgi apparatus and mitochondria, while the representation of RER was similar in neurons of the two shapes. These results suggest that smooth contoured GnRH neurons are more actively transcribing message while thorny neurons are more actively engaged in peptide processing and packaging. These differences appear to be independent of synaptic input, but this must be confirmed by chemical identification of the synapses. NIH AG05366.

430.2

SEXUAL DIMORPHISM IN THE INNERVATION OF GnRH NEURONS IN THE RAT.

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The pattern of gonadotropin secretion and presumably the release of GnRH varies between the sexes. To determine if dimorphic neurohormone release might be due to differences in the synaptic input to GnRH cells, we undertook a quantitative study identifying total input as well as two chemically identified inputs, the endogenous opiate beta-endorphin (B-E) and GnRH. Young adult (3-4 mos) Fischer 344 rats (2 male and 4 diestrus II female) were deeply anesthetized with sodium pentobarbital and perfused with 4% paraformaldehyde. Vibratome sections through the diagonal band and preoptic area were treated for the ultrastructural localization of B-E and GnRH immunoreactivity with DAB and TMB as the respective chromogens (Chen et al, Soc. Neurosci. 14:439, '88). In two of the females the antibodies were used in the reverse sequence to verify that there was no bias imposed by the order of the reactions. 20-30 serial ultrathin sections of 20 GnRH neurons (recognized by the presence of large TMB crystals) from the males and 15 from 2 females were photographed and analyzed for the presence of synapses. Synapses were categorized as B-E or GnRH containing, or were "non-identified". Dendrites from the same thin sections were also analyzed. Several categories of synaptic input did show a sexual dimorphism. These included twice as many synapses in total onto the cell soma of females ($p<0.001$) as well as twice as much B-E axosomatic and axodendritic input. Neither the total number of synapses to GnRH dendrites nor the number of GnRH-GnRH interactions varied between the sexes. In both sexes there were occasional GnRH/GnRH dendrosomatic appositions defined by the presence of membrane thickenings and clefts between the two elements, and occasional "synaptic" vesicles. Thus, we have demonstrated that the organization of the synaptic input to GnRH neurons differs between male and female and a portion of the dimorphism is due to the B-E input. China Medical Board (WPC), HD 10665 (AJS).

430.3

DIET RESTRICTION AND LHRH NEURON MATURATION.

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LHRH neurons show changes during postnatal development: their morphology shifts from smooth to irregular and plateaus at the time of puberty. We sought to determine whether the programming of morphological changes in LHRH neurons can be interrupted by delaying the onset of puberty by weight restriction. Female rat pups were fed ad libitum or maintained at 75g body weight from 24 days of age until 35-40 days (normal puberty). A third group of rats were weight-restricted, refed at 40 days, and then sacrificed after vaginal opening (approx. 4-5 days after refeeding). Tissue was prepared by perfusion with acrolein and stained for LHRH using standard immunocytochemical techniques and the LR-1 antibody. Weight restricted, refed and control animals all showed comparable cell numbers for LHRH, indicating that restricted animals had no significant impairments in LHRH cell viability or synthesis. The shift from smooth to irregular contours that normally accompanies postnatal maturation was maintained in the diet-restricted animals. However, pre- and post-pubertal LHRH neurons also differ in the apparent subcellular distribution of LHRH within the cytoplasm. In 24 day old rats, most LHRH cells are characterized by immunoreactivity within the entire cytoplasmic compartment; a small number of cells display distinct granules in the cytoplasm with little extragranular LHRH. In contrast, in the pubertal rats, a greater portion of the cells are distinctly granular. The diet-restricted rats maintained the prepubertal cytoplasmic pattern; refeeding rapidly established the pubertal pattern. These results suggest that granularity can be used to reflect the activity of the LHRH system, and may underlie changes in the processing or storage of LHRH.

430.4

GNRH-, VASOPRESSIN-, AND GAD-IR NEUROENDOCRINE NEURONS INTERACT SYNAPTICALLY IN THE SUPRAOPTIC NUCLEUS OF JUVENILE MONKEYS.

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Vasopressin (VP) and gamma-aminobutyric acid (GABA) are hypophysiotropic hormones also involved in control of gonadotropin-releasing hormone (GnRH) secretion. We examined whether GnRH, VP, and glutamic acid decarboxylase (GAD) immunoreactive (-IR) elements interact synaptically in the supraoptic nucleus (SON) of cynomolgus monkeys. Neuroendocrine (NEU) neurons in 4 juveniles were retrogradely labeled before aldehyde perfusion (see Soc. Neurosci. Abstr. 14:439, 1988). Frontal vibratome sections were each immunostained for GnRH with PAP, VP with 5 nm gold, and GAD with 15 nm gold. Most of the VP-IR and the few GnRH-IR cell bodies in the SON were NEU. VP-IR elements formed axodendritic and axosomatic symmetrical synapses (SS) with one another. Serial thin sections revealed SS between GnRH axon terminals and both VP-IR dendrites and NEU cell bodies. Conversely, VP-IR boutons also formed SS with NEU GnRH-IR neurons. Of 312 VP and GAD profiles, 38% were -IR for both VP and GAD. VP+GAD-IR axons made asymmetrical synapses with GAD-IR dendrites (suggesting coexistence of an unidentified transmitter), but did not synapse with GnRH-IR elements. Longi-cut VP+GAD-IR axons and somata had regions where either VP-IR or GAD-IR was dominant, the significance of which is unclear. Our study demonstrates anatomical synapses between GnRH-IR and VP-IR neurons in SON, in which postsynaptic neurons were NEU. The presence of VP+GAD-IR axons also suggests coordinated roles for VP and GABA in neuromodulation or neuroendocrine control. (Supported by NIH HD10907).

430.5

EVIDENCE FOR PROCESSING INTERMEDIATES IN THE METABOLIC PATHWAY FROM PRO-LHRH TO LHRH. W.C. Wetsel, C.A. Johnston, and A. Negro-Vilar. Reprod. Neuroendo. Sect., Lab. of Mol. and Integrative Neurosci., NIEHS, Res. Tri. Pk., NC 27709.

Previous studies from our laboratory suggest that the molar ratio of gonadotropin-releasing hormone-associated peptide (GAP) to LHRH is variable in cell bodies, fibers, and nerve terminals during the estrous cycle of the rat. In the present study, we have evaluated these changes in peptide levels during noon of diestrus 2. Punches from the medial preoptic nucleus (MPO), diagonal band of Broca (DBB), retrochiasmatic area (RCA), arcuate nucleus (AN) and median eminence (ME) were collected from 90 rats. Materials, that were separated according to molecular weight, were screened both for GAP-like immunoreactivity LI) with MC-2 antisera and for LHRH-LI with A772 and Rice #5 antisera. A concentration gradient of precursor to products was found from the cell body to the nerve terminal region(s). The MC-2 antisera detected materials in the MPO and DBB at approximately 14000-16000, 8500, and 6700 molecular weight, while the ME only contained the latter 2 forms. At least 3 different molecular forms of LHRH were bound in the MPO, DBB and RCA, while the AN and ME contained only mature LHRH. These data suggest that at least 3 additional post-translational steps must occur before the mature LHRH is produced from pro-LHRH. The regulation of these processing steps may have important functional consequences during the estrous cycle.

430.6

POSTNATAL DEVELOPEMENT OF proGnRH-GAP GENE EXPRESSION IN THE FEMALE AND MALE RAT BRAIN. M. Jakubowski*, M. Blum and J.L.Roberts. (SPON: V. Friedrich). Fishberg Center for Neurobiology, Mt. Sinai School of Medicine, CUNY, New York, NY 10029

The content of proGnRH-GAP mRNA in the rat preoptic area-anterior hypothalamus (POA-AH) was followed from postnatal day 15 through day 63. Sprague-Dawley rats were sacrificed by decapitation and cytoplasmic RNA fractionated from individual POA-AH homogenates was purified using proteinase K digestion. Cytoplasmic proGnRH-GAP mRNA was quantitated along with cyclophilin mRNA (an internal standard control) using solution hybridization-RNase protection assay. Cyclophilin mRNA levels normalized against the content of total RNA proved to remain constant across the different age groups (p = 0.3). However, proGnRH-GAP mRNA showed a significant sex-related increase with age (p = 0.0001). In females, the mean fg proGnRH-GAP mRNA/μg total RNA levels increased from 1.7, 3.0, 4.8, 3.8 and 3.7 on days 15, 20, 25, 29 and 34, respectively, to 7.2 and 15.5 on days 44 and 63, respectively. In males, the mean fg proGnRH-GAP mRNA/μg total RNA levels increased from 2.3 and 3.5 on days 15 and 20, respectively, to 6.2-7.3 in the older age groups.

These results show a sex difference in the postnatal development of proGnRH-GAP gene expression in the rat POA-AH. In males, proGnRH-GAP mRNA levels increase around the time of weaning (day 25) and remain unchanged thereafter. In females, proGnRH-GAP mRNA stores increase at the time of puberty (day 44) to levels shown by adult males, with a further 2-fold increase in adulthood.

430.7

DO CATECHOLAMINERGIC, NEUROPEPTIDE Y, SUBSTANCE P, AND GABAERGIC TERMINALS INNERVATE GONADOTROPIN RELEASING HORMONE (GNRH) NEURONS IN THE SHEEP? F.J. Karsch*, F.J. P. Ebling*, & M.N. Lehman. Reprod. Sci. Prog., Univ. Mich., Ann Arbor, MI; Dept. Anat. & Cell Biol., Univ. Cincinnati Coll. Med., Cinti, OH.

Pharmacologic evidence suggests that neurons which secrete GnRH are regulated by a wide variety of neurotransmitter systems. To unambiguously determine the neurochemical identity of synaptic inputs onto GnRH neurons in sheep, we used a double label immunocytochemical protocol which yields visibly different reaction products at both light and electron microscopic (EM) levels (Norgren & Lehman, *J. Histochem. Cytochem.*, in press). Suffolk ewes (n=12) were perfused intracranially during mid-breeding season and potential contacts examined between immunoreactive tyrosine hydroxylase (TH), neuropeptide Y (NPY), substance P (SP), or glutamic acid decarboxylase (GAD) terminals and GnRH neurons in the preoptic area and anterior hypothalamus. At light and EM levels, NPY-, SP-, and GAD-positive varicosities were more frequently apposed to GnRH somas and dendrites than were TH-positive boutons. In all thin sections, non-identified terminals contacting GnRH neurons were more numerous than immunoreactive ones. TH-, NPY-, and SP-positive axon terminals contained clear spherical vesicles (20-40 nm diam.) and larger dense-core vesicles (80-120 nm diam.); GAD terminals contained only small clear vesicles. Thus far we have seen synaptic modifications only between NPY and SP terminals and GnRH dendrites. [Supported by NIH grants HD 18337 (FJK) and HD 21968 (MNL)]

430.8

GONADOTROPIN RELEASING HORMONE (GnRH) FROM THE AMNIONIC FLUID BINDS TO GnRH RECEPTORS IN THE DEVELOPING RAT PITUITARY. L. Jennes. Dept. Anatomy, Wright State Univ. Sch. of Med., Dayton, OH 45435.

With *in vivo* and *in vitro* autoradiography, specific GnRH receptors were identified in the developing pituitary as early as at fetal day E13. Immunohistochemistry and radioimmunoassay (RIA) could not detect GnRH in fetal (E12-E14) brains, however, this peptide could be measured in the amnionic fluid (AF). GnRH levels in the AF were low at day E12 (0-30 pM/ml), they increased at days E15-16 to 250 pM/ml before they declined to 100 pM/ml at days E17-20. The identity of GnRH in the AF was verified by a) RIA of AF extracts using 3 different anti-GnRH antibodies, b) gel filtration of AF extracts followed by RIA and c) the ability of AF to stimulate LH release in pentobarbital anesthetized rats. Since ^{125}I-Buserelin injected intravenously into the maternal circulation can cross the fetal-placental barrier and since Buserelin injected into the amnionic fluid binds to fetal pituitary GnRH receptors, it is suggested that maternal GnRH from the placenta reaches the fetal pituitary and may play an important role in the development of fetal gonadotropes.

430.9

CO-EXISTENCE OF LUTEINIZING HORMONE RELEASING HORMONE (LHRH) AND GALANIN IN A SUBPOPULATION OF NEURONS IN THE PREOPTIC REGION OF THE RAT BRAIN. I. Merchenthaler* and A. Negro-Vilar (SPON: L.C. Towns). Lab. of Mol. Integrat. Neurosci., NIEHS/NIH, Res. Triangle Park, NC 27709

The presence of galanin (GAL) fibers and terminals in the median eminence of the hypothalamus suggests an involvement of GAL in neuroendocrine functions. GAL is broadly distributed and co-localized with several peptides and amines. The distribution of GAL and LHRH perikarya within the preoptic area (POA) is similar. Although the majority of GAL neurons are round and multipolar, a certain subpopulation of neurons in the POA are fusiform. The appearance of these neurons is similar to the typical LHRH nerve cells. Double labeling immunocytochemistry on paraffin and vibratome sections from rat brains revealed that a certain subpopulation of LHRH perikarya in the POA is also immunoreactive for GAL. Each of three major technical approaches, i.e. "adjacent section method", the "elution-restaining technique" and the "direct double-staining technique" indicated that a subpopulation of LHRH neurons in the POA co-express GAL immunoreactivity. This represents the first demonstration of co-localization of another peptide in an LHRH neuron. Co-localization of GAL with LHRH in the same perikaryon suggests that GAL (a) may influence the release of LHRH; (b) may participate in the autoregulatory mechanism of LHRH secretion; (c) may be co-released and interact in a cooperative mannner with LHRH on pituitary gonadotropes.

430.10

PITUITARY FUNCTION IN THE DEVELOPING MALE TREE SHREW (TUPAIA BELANGERI). P.M. Collins*, W.N. Tsang*, and H. Urbanski. Dept. of Biol. Sci., Univ. of California, Santa Barbara, CA 93106.

The pattern of postnatal development in the male tree shrew conforms with the general primate pattern yet occurs within a condensed time-frame (90 days) which allows the establishment of precise endocrine correlates of reproductive events (Collins, P.M. and Tsang, W.N., Biol. Reprod. 37:261, 1987). To evaluate the role of gonadotropins in gonadal development FSH and LH were determined by RIA from birth to sexual maturity. Both gonadotropins remained low during the infantile phase (birth to Day 30) varying within narrow limits. FSH rose continuously between Days 40-45 and Days 66-75 in association with the onset of spermatogenesis and the accelerated growth of the testis (> 2-fold increase, $p<0.05$). FSH levels then declined in relation to the appearance of spermatids and the progression of spermiogenesis. LH levels were higher in pubertal as compared with infantile animals ($p<0.05$) with initial elevations coinciding with maximum plasma testosterone levels (Days 50-55). The data suggest a role for FSH and LH in the establishment of the gametogenic and endocrine functions of the testis respectively. As in Man and other primates, puberty in the tree shrew follows an extended infantile phase and appears to be a response to the activation of the hypothalamo-pituitary axis.

430.11

COMPUTER 3D RECONSTRUCTION OF MEDIAN EMINENCE MICRO-CAPILLARIES AT HIGH RESOLUTION. L.S. Hibbard[1], B.J. Dovey-Hartman[2*], R.B. Page[2], [1]Washington University School of Medicine, St.Louis, MO 63110, and [2]The Pennsylvania State University College of Medicine, Hershey, PA 17033.

The microvasculature of the median eminence (ME) of the hypothalamus modulates hormonal communication between the brain and the pituitary. We are now reconstructing ME capillary loops with the aim of locating the cellular apparatus responsible for blood flow control. These loop structures were selected after examining the 3D reconstruction of a low magnification study of this same tissue block, reported earlier (Soc. Neurosci. Abstr., 14:630, 1988). In that study, we found several capillary structures projecting from the outer to the inner plexus of the ME, like those observed in previous work (Page, et al., Am. J. Anat., 146:273, 1976).

We are now reconstructing discrete capillary structures with attached cellular elements from TEM images at 1650X. Digital image processing techniques are being used to create mosaics of overlapping images on each level, and to align the resulting mosaics (Hibbard, et al., Comput. Bio. Res., 16:411, 1986). Edge detection and tracking on the mosaics will provide intercellular boundaries, and local texture measures may be used to denote intracellular contents. (Support: NSF BNS-8506479 and NIH NS15926.)

430.12

DOPAMINE DOES NOT PLAY A ROLE IN THE REGULATION OF BLOOD FLOW IN THE OVINE MEDIAN EMINENCE. R.B.Page,M.Gropper*, E.Woodard*, J.Townsend*,S.Davis* and R.Bryan. Dept. of Surgery (Div. of Neurosurgery) Milton S. Hershey Medical Center of the Pennsylvania State Univ., Hershey, PA 17033.

Blood flow was measured in the ovine median eminence and neural lobe prior to and after the intravenous infusion of dopamine (N=6), the D_1 angonist SK&F 38393 (n=4), the D_2 agonist bromocriptine (N=4), and the dopamine antagonist haloperidol (N=5). It was also measured prior to and following the intracarotid infusion of dopamine into 8 naive sheep and 7 sheep pretreated with phenoxybenzamine. Radiolabelled microspheres were used to determine regional cerebral (RCBF) and regional neurohypophysial blood flow (RNHBF) as well as blood flow in the choroid plexus and kidneys in these 34 adult female sheep anesthetized with pentobarbital. Samples for serum prolactin measurement by radioimmunoassay were obtained before and after drug infusion. Haloperidol caused a significant fall in blood flow in median eminence (from 560±90 to 379±66 ml/100gm/min [mean±SE]) as well as in neural lobe, choroid plexus, caudate nucleus and kidneys. Intravenous dopamine infusion increased renal and choroid plexus blood flow but did not change RNHBF. Intracarotid dopamine infusion decreased median eminence and neural lobe blood flow and increased choroid plexus blood flow. Pretreatment with phenoxybenzamine only partially blocked the vasoconstrictor effect of dopamine. These findings do not support a role of dopamine in the regulation of median eminence blood flow.

430.13

GLUTAMATE IMMUNOREACTIVITY IN THE RAT NEURAL LOBE: AN ULTRASTRUCTURAL ANALYSIS OF NEUROSECRETORY ENDINGS AND PITUICYTES. R.B. Meeker, D.J. Swanson*, R.S. Greenwood and J.N. Hayward, Neurobiology Curriculum and Dept. of Neurology, University of North Carolina, Chapel Hill, NC 27599.

To explore the possibility that the high level of glutamate immunoreactivity which we have previously observed in the neuroendocrine cell bodies of the supraoptic nucleus might represent a neurotransmitter pool of glutamate, we examined the distribution of glutamate immunoreactivity within the neurosecretcry terminal field in the neural lobe. Rat brains fixed by perfusion with 4% paraformaldehyde, 2% glutaraldehyde and 15% saturated picric acid in 0.1 M cacodylate buffer were cut on a vibratome and stained with a rabbit polyclonal antiserum to glutamate-hemocyanin. Both pre-embedding (1:5000 Ab, ABC technique) and post-embedding (LR White, 1:10,000 Ab, 10-15 nm goat anti-rabbit collidal gold) staining revealed a high level of glutamate immunoreactivity associated with pituicytes. Little or no immunoreactivity was found in endothelial cells, phagocytic structures or astroglia with the hypothalamus. Neurosecretory axons or swellings rarely stained while the neurosecretory terminals exhibited a low level of staining over the population of small clear vesicles. Two days of water deprivation increased the level of glutamate immunoreactivity in the pituicytes and neurosecretory endings. These observations suggest that glutamate might be used as a transmitter by the neurosecretory endings although the levels of glutamate are very low. Glutamate in the pituicytes may reflect a number of possible functions including: 1) uptake of extracellular glutamate, 2) neutralization of ammonia, 3) active GABA sythesis or 4) a specialized pool associated with local osmoregulation.

Supported by NIH Javits Award NS-14311.

430.14

ULTRASTRUCTURAL ANALYSIS OF NEUROPEPTIDE Y IMMUNOREACTIVITY IN THE SUPRAOPTIC AND PARAVENTRICULAR NUCLEUS OF THE RAT HYPOTHALAMUS. D.J. Swanson*, R.B. Meeker, J.N. Hayward, Neurobiology Curriculum and Dept. of Neurology, University of North Carolina, Chapel Hill, NC 27599.

High concentrations of immunoreactive neuropeptide Y (NPY) varicosities exist in the periventricular nucleus, paraventricular nucleus (PVN), the median eminence (ME) and basomedial hypothalamus with lower levels in the supraoptic nucleus (SON). Within these regions NPY may function to control local blood flow (Edvinsson, et al., 1987) and/or regulate vasopressin secretion (Swanson, 1987). To more clearly understand the role of this peptide we examined the ultrastructure of the terminal fields in the SON, PVN and ME. The brain of male Sprague-Dawley rats was fixed by perfusion with 4% paraformaldehyde, 0.1% glutaraldehyde, 15% saturated picric acid in 0.1 M phosphate buffer, pH 7.4. Vibratome sections were cut at 50 um and stained utilizing a polyclonal antiserum to neuropeptide Y (1:2000) and the double-bridge PAP technique. We observed a high density of large terminal endings containing clear round and dense-cored vesicles making axodendritic or, in a few cases, axosomatic contact with cells in the parvocellular vasopressin and oxytocin subdivisions of the PVN and periventricular hypothalamus. A small number of terminals was observed in the SON, almost exclusively localized to the ventral dendritic neuropil. In the ME, little evidence was found for a direct interaction with neurosecretory axons. No evidence was found for direct interaction with blood vessels within these regions. We conclude that hypothalamic NPY terminals may provide extensive direct afferent input to vasopressin and oxytocin neurons in the PVN as well as more modest levels of input to the SON

Supported by NIH Javits Award NS-14311.

430.15

HORMONAL CONTROL OF NPY IN THE SEXUALLY DIMORPHIC HYPOGASTRIC GANGLIA. B. SCHROEDER* AND R. W. HAMILL (SPON: M. Mangiapane). MONROE COMMUNITY HOSPITAL/UNIV. OF ROCH. ROCHESTER, N. Y. 14603

Gonadal steroids regulate the ontogeny and adult levels of tyrosine hydroxylase (TH) in sexually dimorphic hypogastric ganglia (HG). Neonatal castration (Cx) precludes neurochemical maturation (TH values are <10% of controls), and adult Cx decreases TH activity 85% by one month. Neuropeptide Y (NPY) is co-contained within peripheral noradrenergic neurons. The present studies test the hypothesis that hormones regulate NPY in HG and in the vas deferens (VD), a major target organ of the HG. Adult Sprague-Dawley rats were Cx'd at 70 days of age and NPY, TH and NE examined in HG and VD after surgery (animal surgery and care followed strict NIH guidelines) . Following Cx., NPY levels gradually declined: values of 84%, 60% and 30% of control at 1, 2, & 4 weeks (wks) respectively. TH and NE followed a similar profile. VD, NPY was unchanged 1 wk following Cx, but declined 35% by 2 wks and 59% by 4 wks. TH declined more rapidly but stabilized at a similar nadir (40% of control). T replacement fully corrects the observed alterations. NPY data expressed per gram wet weight of tissue reveal no significant decrease in NPY, suggesting target organ regulation of ganglion peptide content. These studies expand sexual dimorphic aspects of ganglia and indicate that gonadal steroids exert regulatory efects on NPY in sympathetic ganglia and target tissues.

430.16

TEMPORAL RELATIONSHIPS BETWEEN 17β-ESTRADIOL, LUTEINIZING HORMONE AND GALANIN DURING SEXUAL MATURATION IN THE FEMALE RAT. S.M. Gabriel, Department of Psychiatry, Mount Sinai School of Medicine, New York, NY 10029.

Recent studies suggest that the 29-amino acid peptide galanin is potently regulated by estrogen in neuroendocrine tissues of the rat (*Kaplan, Gabriel, Koenig et al, PNAS 85:7408-7412, 1988*). This estrogenic influence is first expressed during puberty when concentrations of galanin-like immunoreactivity (galanin-LI) increase in the anterior pituitary (AP), neurointermediate lobe (NIL) and median eminence (ME) of female rats relative to male rats. To evaluate the temporal relationship between estrogen and galanin during this period of development, prepubertal female littermates were observed daily for evidence of vaginal opening, as an indication of pubertal onset and the first proestrus luteinizing hormone (LH) surge. Animals were sacrificed between 1700 and 1900h when 30% of the rats showed vaginal opening. Concentrations of galanin-LI were measured by a newly developed radioimmunoassay specific to rat galanin. Elevated serum 17β-estradiol and LH concentrations preceded vaginal opening while elevated galanin-LI concentrations in the ME, NIL and AP followed vaginal opening. A similar temporal sequence of 17β-estradiol, LH and galanin-LI concentrations were noted in peripubertal female rats 1, 2 and three days after injection with pregnant mare serum gonadotropin *(PMSG, 10 IU, s.c., 1000h)*. The effects of PMSG were probably due to increased 17β-estradiol secretion, because galanin-LI did not increase in prepubertal male or ovariectomized female rats following PMSG injection. These studies indicate that galanin-LI is influenced by circulating estrogens at puberty in the female rat. They further suggest a role for galanin in sexual maturation of the neuroendocrine axis.

430.17

GONADAL STEROIDS MODIFY DENDRITIC SPINES IN HYPOTHALAMIC NEURONS: A GOLGI STUDY IN THE ADULT RAT. M. Frankfurt E. Gould* and B.S. McEwen Neuroendocrinology Lab, The Rockefeller University, New York, NY 10021

A golgi study of neurons in the adult rat ventromedial (VMN) and dorsomedial (DMN) hypothalamic nucleus was done in intact male and female rats and ovariectomized (OVX) rats treated with oil (O), estrogen (E) or E and progesterone (P). Golgi-impregnated neurons were drawn and then analyzed using SMI morphometry to determine possible differences in cell body size, number of primary dendrites, number of branchpoints and spine density. Statistical differences were determined using 1 way ANOVA followed by the Neuman-Keuls test ($p<.05$). In the VMN of OVX rats given E alone or E in conjunction with P, there was a significantly greater density of dendritic spines than in the OVX-O group. There were no sex differences in any parameter in the VMN. In the DMN, the OVX-E-P group contained a significantly greater density of dendritic spines than the OVX-O group. Moreover, in the DMN of intact males there was a greater density of dendritic spines than in the intact females. There were no differences in cell body size, number of primary dendrites or number of branchpoints in either the VMN or DMN. From these results it appears that steroid feedback can result in short-term morphological changes in the hypothalamus.

Supported by MH41256

430.18

IMMUNOCYTOCHEMICAL LOCALIZATION OF AROMATASE IN THE QUAIL BRAIN

J. Balthazart and A. Foidart *, Laboratory of General and Comparative Biochemistry, University of Liège Belgium.

The presence of aromatase in the brain of vertebrates was detected a long time ago. In many studies, the activity of the enzyme was studied by in vitro radioenzyme assays. These showed that the enzyme has a discrete localization in the brain and that its activity is modulated by the physiological condition of the animal. Factors such as age, sex, castration or steroid therapy all affect enzyme activity. However, the precise neuroanatomical localization of the enzyme and the mechanisms underlying the activity changes (modification of enzyme concentration or regulation of enzyme activity) have not been determined so far. By a peroxidase-antiperoxidase immunocytochemical method using a polyclonal antibody raised against human placental aromatase and purified by affinity chromatography, we have for the first time successfully localized aromatase containing cells in the brain of a vertebrate, the Japanese quail. The specificity of the immunocytochemical reaction has been demonstrated. The cytoplasmic localization of the enzyme is confirmed. It is found primarily in neurons but double-labelling experiments using specific neuronal or glial markers should be performed to confirm this conclusion. Aromatase-positive cells are detected in all areas which were shown previously to contain aromatase activity by product formation assays, namely the preoptic area (POA) and various hypothalamic and septal nuclei. Within these areas, the distribution of the immunoreactive cells is however heterogeneous. In particular, within the POA most if not all positive cells are found in the sexually dimorphic preoptic medial nucleus (POM). In the POM, the number of immunoreactive cells and the intensity of their staining are drastically decreased by castration and restored to levels typical of sexually mature males by testosterone treatment. This demonstrates that the induction of aromatase activity which is caused by testosterone reflects real changes in enzyme concentration (enzyme induction) rather than a modulation in the activity of a constant number of molecules.

430.19

DISTRIBUTION OF β-ENDORPHIN IMMUNOREACTIVITY IN THE ARCUATE NUCLEUS AND SURROUNDING HYPOTHALAMUS OF THE FEMALE GUINEA PIG. J.E. Thornton, M.D. Loose*, M.J. Kelly and O.K. Ronnekleiv, Dept. Physiol., OHSU, Portland, OR 97201 and ORPRC, Beaverton, OR 97206.

Although β-endorphin (β-end) may play an important role in neuroendocrine function of the guinea pig, the distribution of β-end in the guinea pig brain has not been examined. Guinea pigs were ovariectomized, and one week later were injected with 25μg estradiol benzoate or oil. Animals were perfused 24h later with 4% paraformaldehyde. Brain sections (50μm) were cut on a vibratome. β-End was localized using avidin-biotin-HRP immunocytochemistry (Eskay BE2 antisera). No cell bodies were seen rostral to the retrochiasmatic area (RCA). In the RCA, cells (10-50/section) were seen lying basomedially and extending laterally. In the rostral ARC, cells were concentrated (100-200/section) beneath the third ventricle (IIIV) and extended dorsolaterally. As the IIIV descended and the median eminence formed, the cells separated into bilateral groups which lay in the basolateral part of the ARC and extended into the cell poor zone. In the central ARC, cells shifted more dorsomedially and extended dorsally into the periventricular part of the ARC. In the caudal ARC, cells again formed two basolateral clusters and were also seen along the floor of the IIIV. Cells were not seen in other parts of the hypothalamus. Preliminary data indicate that estrogen increased the number of β-end immunoreactive cells. (PHS DA 05158, HD16793)

HYPOTHALAMUS

431.1

EFFERENT CONNECTIONS OF THE ANTERIOR HYPOTHALAMIC AREA (AHA) STUDIED WITH THE NEW ANTEROGRADE TRACER PHA-L. J.M. Wyss and Th. van Groen. Department of Cell Biology and Anatomy, University of Alabama at Birmingham, Birmingham, AL 35294.

The projections arising in the anterior hypothalamic area (AHA) have been characterized previously using the autoradiographic method for detection of anterogradely transported [^{3}H] amino acids. In the present experiments we have employed the PHA-L method to determine the specificity of these projections in more detail. The PHA-L method facilitates the distinction between fibers of passage and synapsing fibers. Iontophoretic injections were made stereotaxically in the AHA. Following the immunohistochemical staining of the transported PHA-L using the PAP-method, the location of labeled cell bodies, axons and their terminals were mapped. Rostral to the AHA, labeled axons appeared to terminate in the medial preoptic area, the ventrolateral quadrant of the lateral septal nucleus, the horizontal limb of the diagonal band of Broca, and the medial part of the nucleus accumbens. Caudally projecting axons labeled by the AHA injections terminated ventromedial in the lateral and posterior hypothalamic areas, the periventricular and dorsomedial nuclei of the hypothalamus, the supramamillary region, the ventral part of the medial mamillary nucleus, the paraventricular nucleus of the thalamus, the amygdala, and the periaquaductal gray. A few labeled axons also were present in the raphe nuclei, the locus coeruleus and the nucleus solitarius. Finally, labeled fibers were present in the hippocampal formation, mainly in area CA_3 and the subiculum, where a relatively heavy projection was present in the temporal part of the hippocampus. Contralaterally, fibers were labeled in the lateral septal nucleus, the AHA, the periventricular nucleus of the hypothalamus, the ventromedial hypothalamus, the paraventricular nucleus of the thalamus, the central gray, and the nucleus solitarius.

431.2

HYPOTHALAMOSPINAL TRACT: QUANTITATIVE COMPARISON IN 24 SPECIES. D. P. Sutherland*, R. J. Nudo and R. B. Masterton (SPON: F. K. Stephan). Dept. Psychology, Florida State Univ., Tallahassee, FL 32306.

We have labeled the cell groups projecting to the spinal cord in 23 mammals and one reptile by hemisecting the spinal cord at the C1-C2 junction, applying raw HRP to the cut axons and then processing the resulting materials with TMB. After counting the labeled cells in the hypothalamus and correcting stereologically for overcounting, the variation in number was subjected to strict statistical analyses for its covariation with several non-neurological, neurological, historical, and ecological characteristics of the animals.

The results show that the total number of hypothalamospinal neurons is entirely unrelated to brain size, body size, or neocortical surface area though it is weakly related to the number of corticospinal, rubrospinal, or tectospinal neurons. Arranging the animals on the basis of their propinquity with mankind suggests that the absolute size of the tract probably remained nearly constant throughout the mammalian segment of the Anthropoid lineage (though it probably increased in the Carnivora lineage). Among 8 ecological characteristics tested, the tract is most closely related to the host's food preference with herbivores having the smallest, carnivores the largest. Supported by NIH-NINCDS # NS7726.

431.3

PEPTIDERGIC AFFERENTS TO THE PARAVENTRICULAR NUCLEUS OF THE HYPOTHALAMUS. M.M. Moga and C.B. Saper. Depts. of Pharm. & Physiol. Sci. and Neurology, University of Chicago, Chicago, IL 60637.

The paraventricular nucleus of the hypothalamus (PVH) contains large numbers of afferent fibers that are immunoreactive for a variety of neuropeptides. To determine the significance of these inputs, it will be necessary to identify their origins. We examined the chemical specificity of forebrain afferents to the PVH using six neuropeptide antisera combined with fluorescent retrograde tracing. After Fluorogold injections into the PVH, small numbers of retrogradely labeled neurons were found to be *enkephalin*-like immunoreactive (-ir) in the dorsal part of the lateral septum; *substance P*-ir in the lateral septal nucleus and the medial preoptic area; *somatostatin*-ir in the ventral lateral septal nucleus; *corticotropin releasing factor*-ir in the preoptic portion of the bed nucleus of the stria terminalis, the adjoining lateral preoptic area, and the median preoptic nucleus; *brain natriuretic peptide*-ir in the tuberomammillary nucleus, particularly its medial, posterior periventricular portion; and *neurotensin*-ir in the anteroventral periventricular and median preoptic nuclei. None of these peptides, however, accounted for more than a small percentage of the forebrain afferents to the PVH. Furthermore, immunoreactive cell bodies for each peptide were seen in the PVH, so that part of the peptide-ir innervation may be intrinsic.

431.4

TRANSFORMING GROWTH FACTOR α (TGFα) mRNA IS EXPRESSED IN THE DEVELOPING HYPOTHALAMUS AND TGFα STIMULATES LUTEINIZING HORMONE-RELEASING HORMONE (LHRH) RELEASE. S.R. Ojeda, H.F. Urbanski, M.E. Costa*, D.F. Hill* and M. Moholt-Siebert*. Division of Neuroscience, OR Regional Primate Res. Ctr., Beaverton, OR 97006.

Little is known about the presence of trophic factors in the hypothalamus and the role they may play in regulating the functional development of hypothalamic neurons. We have examined the ability of epidermal growth factor (EGF) and TGFα to affect the release of LHRH, the peptide that controls reproductive development. We have also determined whether the genes encoding EGF and TGFα are expressed in the prepubertal hypothalamus. Median eminences from 28-day-old juvenile female rats were incubated in vitro in the presence of one of several trophic factors. Both EGF and TGFα (2-100 ng/ml) elicited a dose-related increase in LHRH release. TGFβ_1 and β_2 were ineffective. Northern blot analysis of poly A+ RNA utilizing a single-stranded cDNA probe failed to reveal the presence of EGF mRNA in the hypothalamus at any age studied (fetal day 18 to postnatal day 36). In contrast, both an antiRNA probe and a double-stranded cDNA complementary to TGFα mRNA recognized a 4.5 Kb hypothalamic mRNA species identical in size to TGFα mRNA. Levels of expression were highest on fetal day 18 and declined gradually thereafter. The results indicate that: a) EGF and TGFα release LHRH through a non-genomic mechanism, b) since EGF and TGFα share a common receptor, their effect on LHRH is probably mediated by the same receptor, and c) TGFα rather than EGF is the physiological ligand for this interaction. (Supported by NIH grant HD-09988, Project IV).

431.5

DEVELOPMENT AND CHARACTERIZATION OF MONOCLONAL ANTIBODIES DIRECTED AGAINST LHRH. H.F. Urbanski and J. Hackett* (SPON: C.L. Bethea). Division of Neuroscience, Oregon Regional Primate Research Center, Beaverton, OR 97006.

Monoclonal antibodies (MAbs) against luteinizing hormone-releasing hormone (LHRH) were developed for immunocytochemical (ICC) purposes. In essence, LHRH was conjugated to bovine thyroglobulin and 50 μg injected into the spleen of a BALB/c mouse. After 3 weeks, the animal received a second injection (25 μg) and killed 3 days later. The mouse spleen cells were fused with SP2/0 myeloma cells and grown in HAT selective medium. Surviving hybridoma colonies were screened by radioimmunoassay and out of a total of 92, 4 were found to be secreting LHRH antibodies of the IgG_1 sub-class. The secreted MAbs were purified by precipitation with 50% ammonium sulphate solution followed by affinity chromatography using Bakerbond ABx. Two of these LHRH MAbs were subsequently characterized in detail. MAb HU4H showed conformational specificity to both mammalian and chicken-I LHRH while MAb HU11B showed sequential specificity to the C-terminal of mammalian but not chicken-I LHRH. Neither HU4H or HU11B cross reacted with other brain peptides. Moreover, in PAP ICC these pure, specific MAbs labeled LHRH neurons in hamster hypothalami with little background staining.
(supported by NIH grants HD-24312 and RR-00163)

431.6

ANGIOTENSIN (1-7) IMMUNOREACTIVITY IN THE RAT HYPOTHALAMUS C.H. Block, H. Vilsack *, and C.M. Ferrario. Research Institute of the Cleveland Clinic Foundation, Cleveland, OH 44195-5070

We have recently reported the immunocytochemical (ICC) distribution of a novel heptapeptide related to angiotensin (Ang) II in the hypothalamo-neurohypophyseal system (HNS) of the rat (Block, et al. Peptides,1988). Ang (1-7) was localized in neurons of the suprachiasmatic (SCN), paraventricular (PVN), and supraoptic (SON) nuclei and the bed nucleus/stria terminalis (BNST). Immunoreactive (ir) fibers were observed in the paraventriculo-neurohypophyseal tract and in the neurohypophysis. To establish a potential role of Ang (1-7) in fluid homeostasis, ICC studies were conducted in normally-hydrated rats; and 24-48 hr water-deprived and rehydrated rats.

In water-deprived rats, Ang (1-7)-ir found in PVN, SON, SCN, and BNST was similar to the control and rehydrated animals, however the intensity of staining was diminished within PVN and SON. Fibers of the median eminence, infundibular recess, and neurohypophysis contained Herring bodies and staining was diminished in dehydrated animals. To investigate the projections of hypothalamic Ang (1-7), True Blue was microinjected into the pituitary.

Many neurons within the SON and PVN contained True Blue, while only a small population of these retrogradely-labeled cells also contained Ang (1-7). These results indicate that Ang 1-7-ir in the pituitary originates, in part from neurons in the SON and PVN; however the possibility that the dense hypothalamic Ang (1-7) fibers project to other sites, such as brainstem and limbic forebrain, cannot be excluded. These findings coupled with the plasticity of Ang (1-7)-ir in the HNS of animals with altered fluid balance suggests a contribution of this heptapeptide in fluid homeostasis.
(NIH HL-37927, HL-6835, and George Storer Fund)

431.7

GABA: A DOMINANT NEUROTRANSMITTER IN MEDIAL HYPOTHALAMUS. C.Decavel* and A.N.van den Pol. (SPON: W.F.Collins) Sect. Neurosurgery, Yale Med.Sch., New Haven, Ct 06510.

Post-embedding colloidal gold EM immunocytochemistry was used to study the density and organization of immunoreactive axons with characterized GABA antisera in the rat suprachiasmatic, arcuate, paraventricular, and supraoptic nuclei. That the method was reliable was indicated by the finding that different GABA antisera stained the same boutons on serial sections. 3-D reconstruction with serial ultrathin sections through GABA immunoreactive terminals consistently revealed a population of dense core vesicles, often lateral to the synaptic cleft, suggestive of common co-localization of peptides with GABA. The synaptic characteristics of GABAergic axons were studied in the PVN with sections alternatively stained for GABA or for conventional EM. Successive boutons from the same GABAergic axon sometimes made repeated synaptic contacts with the same post-synaptic neuron. That GABA may play a role in the synchronization of neural activity was supported by the finding that other GABAergic axons made multiple synaptic contacts with dendrites or perikarya of neurons in close proximity to one another in the PVN. The density of gold labeling in immunopositive and negative axons and postsynaptic cells was quantified stereologically. As nearly 50% of all boutons in the medial hypothalamus are immunoreactive for GABA and glutamate decarboxylase, GABA may be considered the dominant neurotransmitter here.

431.8

GABA IN THE HISTAMINERGIC TUBEROMAMMILLARY NUCLEUS. H.Ericson*, A.Blomqvist and C.Köhler. Astra Research Centre, S-151 85 Södertälje, Sweden; Dept. of Cell Biology, Univ. Linköping, Fac. Health Sci., S-531 85 Linköping, Sweden.

The organization of GABAergic elements in the tuberomammillary nucleus was examined by using antibodies against gamma aminobutyric acid (GABA) and light and electron microscopy. Most neuronal perikarya of the tuberomammillary nucleus were GABA immunoreactive (GABA-i) and contained reaction product diffusely distributed throughout the cytoplasm and in addition, the nucleus presented intense immunoreactivity. The morphology of the GABA-i perikarya were similar to the morphology of histaminergic perikarya described previously by Hayashi et al., J. Comp. Neurol., 229:233, 1984. The GABA-i perikarya were contacted by relatively few terminals. The mean bouton covering ratio of GABA-i perikarya was 6.5%, whereas the mean bouton covering ratio for GABA-i dendrites in the tuberomammillary nucleus was 31%. Some of the presynaptic terminals were GABA-i and in the cases were these contacts presented synaptic densities they were always classified as being symmetric synapses. In addition, GABA-i perikarya and dendrites formed close contacts which did not present synaptic specializations. The results of the present study suggest that neurons of the histaminergic tuberomammillary nucleus contain the neurotransmitter GABA. Furthermore, GABA released from terminals in the nucleus may act as modulator of cellular processes within the tuberomammillary nucleus.

431.9

IMMUNOCYTOCHEMICAL ANALYSIS OF THE SUPRACHIASMATIC NUCLEUS IN ORGANOTYPIC CULTURES. H.Gainer, M.Castel[1] and S.Wray, LNC, NINDS, Bethesda, MD 20892,[1]Dept. Zool., Hebrew Univ., Israel, 91904.

Using the slice explant roller culture method for organotypic hypothalamic cultures (Wray et al., Peptides 9:1151-1175,1988), we have begun to study the viablity of rat suprachiasmatic nuclei (SCN) *in vitro*. In order to determine to what extent neurons within the SCN survive, express their appropriate phenotypes and maintain synaptic organization, we have immunocytochemically analysed this structure using light (LM) and electron microscopic (EM) techniques after 18 days in culture. Examination of this nucleus at the LM level showed that the SCN maintains a highly compact organization of neurons, similar to that found *in vivo*. Immunocytochemistry (ICC) revealed numerous neurophysin (NP)- and vasoactive intestinal polypeptide (VIP)-containing neurons within the cultured SCN. Many synaptic configurations onto SCN cell bodies were still present after 18 days *in vitro*. Pre-embedding EM-ICC, for either NP or VIP, resulted in specific immunolabelling of secretory granules within neuronal profiles. We are currently determining whether gastrin-releasing peptide- and GABAergic-containing neurons are present in these cultures after 18 days *in vitro*. The presence of an organotypic distribution of specific neuronal phenotypes and abundant synapses in the SCN after 18 days *in vitro*, indicates that this culture system could be a valuable *in vitro* model for the long-term study of SCN function and metabolism.

Supported, in part, by Grant No. 87-00168 from the U.S.-Israel Binational Science Foundation.

431.10

MONOCLONAL ANTIBODIES WHICH RECOGNIZE SUBSETS OF HYPOTHALAMIC MAGNOCELLULAR NEUROSECRETORY NEURONS IN THE RAT. S.J. Hapner, P.E. Marshall* and C.M. Paden. Dept. of Biology, Montana State University, Bozeman, MT 59717.

In order to determine if neurons of the magnocellular neurosecretory system (MNS) of the rat possess cell-type-specific surface molecules which could play a role in development and plasticity of the MNS, we have attempted to produce monoclonal antibodies (MAbs) against dissociated neonatal MNS neurons or membrane extracts of neonatal neural lobe using the tolerization protocol of Hockfield (Science 237:67, 1987). A large number of antibodies which bind specifically to the neurohypophysis were obtained, and dual-label immunocytochemical characterization of two such MAbs on adult brain revealed that they label primarily vasopressin neurons of the MNS but also bind to a smaller proportion of oxytocin neurons. The binding of one of these MAbs, SON1, has also been observed at the EM level in the neural lobe using pre-embedding peroxidase immunocytochemistry. Reaction product was located immediately subjacent to the axolemma in a subpopulation of neurosecretory axons, and did not appear to be associated with secretory granules. These results indicate that neonatal MNS neurons of the rat contain highly specific molecules which are immunogenic in mice. Further characterization of the antigens revealed by these MAbs is in progress. Rabbit anti-oxytocin and anti-vasopressin were provided by Dr. Gaj Nilaver. Supported by NIH grant NS23642 and RCDA NS01318 to CMP.

431.11

ORGANIZATION OF THE HUMAN SUPRACHIASMATIC NUCLEUS. J.K.Mai* and O.Kedziora* (SPON: European Neuroscience Association). Dept. of Neuroanatomy, University of Düsseldorf, D-4000 Düsseldorf, F.R.G.

Serial 20 um paraffin sections of five human hypothalami were processed by a modified Sternberger PAP-DAB method using antibodies (AB) against neurophysin (NPH), vasopressin (VP), neurotensin (NT), VIP, neuropeptide Y (NPY), somatostatin (SST), myelin-basic-protein (MBP), GFA, and the fucosyl-N-acetyl-lactosamine-(FAL)-epitope. Stereological evaluation and computer-aided reconstruction of discriminated immunoreactive (ir) cells revealed regional segregation except for NPY-positive neurons. As in rodents at least two subnuclei were distinguishable: first, the main area characterized by the presence of NPH- and VP-ir neurons. This area also comprised most of the NT-ir neurons. Second, an area located underneath, in the medial and posterior third of the SCN, where no NPH- but NT-, VIP- and NPY-ir neurons were identified. The FAL-epitope was expressed by neurons which were also positive for NPH and NT, and, in addition, by protoplasmic astrocytes, which were seen tightly packed around the margin of the SCN. Fibers immunoreactive for MBP, finally, could be traced to the optic chiasma, periventricular fiber system, the terminal lamina and the the supraoptic commissures.
Supported by grant from the DFG (SFB200).

431.12

INHIBITORY MECHANISMS OF KAPPA-AGONISTS ON SUPRAOPTIC NEURONS IN RAT HYPOTHALAMIC SLICE PREPARATIONS. K. INENAGA[1]*, H. YAMASHITA[1], K. NAKAO[2]* and H. IMURA[2]*. [1]Dept. Physiol., Univ. Occupational and Environmental Health, Kitakyushu 807, Japan and [2]Dept. Internal Med., Kyoto Univ, Kyoto 606, Japan

Inhibitory mechanisms of endogenous kappa-agonists, leumorphin (LM) and dynorphin (Dyn), were investigated by using intracellular recordings in the supraoptic (SON) neuron of the rat hypothalamic slice preparations. Bath application of LM and Dyn (10^{-7}-$3X10^{-6}$ M) elicited slight membrane hyperpolarization with decreased spontaneous firing rate. The effects were blocked by a selective kappa-antagonist, MR2266. LM and Dyn suppressed excitatory synaptic potentials evoked by focal stimulation dorsolateral to the SON and amplitude of membrane noises. On the other hand, mu- and delta-agonists, morphine and DADLE respectively, did not influence the synaptic potentials. LM and Dyn also decreased duration of action potentials which were prolonged by TEA and Cs. The prolonged Ca spikes were blocked by $MnCl_2$. From these results, we suggest that kappa-agonists inhibit the neural activities of neurosecretory cells in the rat SON, pre- and post-synaptically.

431.13

CHARACTERISTICS OF GUINEA PIG ARCUATE (ARC) NEURONS EXHIBITING A LOW THRESHOLD SPIKE (LTS) OR A TRANSIENT OUTWARD CURRENT. M.J. Kelly, M.D. Loose*, and O.K. Ronnekleiv. Dept. Physiology, OHSU, Portland, OR 97201.

ARC neurons have a higher incidence of LTS when hypothalamic slices are prepared from ovariectomized guinea pigs that are estrogen treated versus oil treated. The current underlying the LTS and the cell types which exhibit this conductance were characterized in current and voltage clamp recordings of 94 ARC neurons. Neurons exhibited an RMP > -50 mV (mean = 62.1±1.8mV); R_{in} > 85 MΩ (mean = 361±26 MΩ); and a fast (Na^+) spike > 50mV with overshoot. 32% of ARC neurons (N=30) had an LTS. The LTS was 6-20 mV in amplitude, 30-120 ms in duration, persisted in TTX (1μM; N=7) and was blocked by 500μM Cd^{2+} or 1mM Co^{2+} (N=4). In voltage-clamp (N=5), voltage steps from holding potentials of -70 to -110mV induced a transient inward current that activated near -70mV and exhibited maximun activation near -50mV. Deinactivation was voltage-dependent and increased from -70 to -110mV. Another 30% (N=28) of ARC neurons exhibited a delayed return to baseline after hyperpolarizing current pulses which in voltage clamp (N=5) was characterized as a transient outward current of up to 500 pA. Its activation/inactivation curves overlapped in the -55 to -70 mV range. Both cell types exhibited time-dependent rectification to hyperpolarizing steps and were hyperpolarized by μ-opioid agonists. A subgroup of the ARC neurons which exhibit an LTS contain tyrosine hydroxylase. (PHS DA 05158, HD 00718)

431.14

OPIOID ACTIONS ON ARCUATE (ARC) NEURONS: A VOLTAGE- AND CURRENT-CLAMP STUDY OF UNIDENTIFIED AND IMMUNOPOSITIVE β-ENDORPHIN AND TYROSINE HYDROXYLASE NEURONS. M.D. Loose*, O.K. Ronnekleiv and M.J. Kelly (SPON: C.J. Russell). Dept. Physiology, Ore Hlth Sci Univ., Portland, OR 97201.

Intracellular recordings were made from ARC neurons in brain slices prepared from ovariectomized guinea pigs pretreated with estradiol. Cells recorded with electrodes filled with biocytin were identified by staining with a streptavidin/FITC complex followed by immunocytochemistry for β-Endorphin (END) or tyrosine hydroxylase (TH). Neurons recorded using biocytin or K-citrate electrodes had similar membrane properties: RMP, -59±1 mV; R_{in}, 355±25 MΩ; τ, 19.6±2.0 msec. The μ-opioid agonist Tyr-D-Ala-Gly-MePhe-Gly-ol (DAGO) induced a decrease in firing and/or a membrane hyperpolarization of 3-21mV in >70% of cells tested (N=88). R_{in} was decreased by 6-57% and the reversal potential was -95±2 mV (N=12). Fifteen cells recorded with either biocytin or K-citrate were voltage clamped. DAGO induced an outward current of 50-180 pA at -60mV which reversed and became inward at potentials negative to -93±2 mV (N=6), near E_k (-94mV). Altering the concentration of K^+ in the medium shifted the reversal potential by 58mV/log unit change in $[K^+]_o$. 47 biocytin-filled cells were characterized morphologically. Both END (N=4) and TH (N=4) immunoreactive cells have been double-labeled and both types were hyperpolarized by DAGO. (PHS DA 05158, HD 16793, 00718).

431.15

ANTAGONISM OF FAST EXCITATORY POSTSYNAPTIC POTENTIALS IN SUPRACHIASMATIC NUCLEUS NEURONS BY EXCITATORY AMINO ACID ANTAGONISTS. Y.I. Kim and F.E. Dudek. Mental Retardation Research Center, UCLA School of Medicine, Los Angeles, CA 90024.

The possible role of excitatory amino acids (EAAs) in fast synaptic transmission in the suprachiasmatic nucleus (SCN) was investigated with intracellular recording. Seven SCN neurons were recorded in horizontal and coronal brain slices prepared from five male rats and two male guinea pigs, respectively. Fast excitatory postsynaptic potentials (EPSPs) were evoked by stimulating optic nerve, optic chiasm ventrolateral to the SCN or a site dorsolateral to the SCN. At resting potential, spontaneous action potentials often obscured the EPSPs. When cells were hyperpolarized 20-40 mV below threshold, depolarizing PSPs from optic nerve had mean amplitude and onset latency of 5.6 mV and 12.3 ms (n=4), while PSPs from other sites were 7.0 mV and 3.0 ms (n=6). Bath-applied kynurenic acid (1 mM), a wide-spectrum EAA antagonist, attenuated these PSPs from optic nerve (n=2) and optic chiasm (n=1) by 21-34%. In slices treated with bicuculline (a $GABA_A$ antagonist, 50 μM), 6,7-dinitroquinoxaline-2,3-dione (0.3-3.0 μM), a non-NMDA receptor antagonist, attenuated the PSPs from optic nerve (n=2), optic chiasm (n=1) and the dorsolateral site (n=2) by 15-76%. The data suggest that EAAs, presumably through non-NMDA receptors, mediate fast excitatory synaptic transmission in the SCN. This includes both retinal and non-retinal input. Supported by AFOSR87-0361.

431.16

COMPARATIVE ELECTROPHYSIOLOGY OF MAGNOCELLULAR AND PARVOCELLULAR NEURONS OF THE HYPOTHALAMIC PARAVENTRICULAR NUCLEUS. N.W. Hoffman*, J.G. Tasker and F.E. Dudek (SPON:R.S. Fisher). Mental Retardation Research Center, UCLA School of Medicine, Los Angeles, CA 90024.

The hypothalamic paraventricular nucleus (PVN) contains both magnocellular and parvocellular neuronal populations, which makes selective intracellular study of either one difficult. Virtually nothing is known about the electrophysiology of PVN parvocellular neurons. We recorded intracellularly from PVN neurons (n=32) in coronal hypothalamic slices. About 60% of recorded neurons displayed Ca^{2+}-dependent low-threshold spikes (i.e., LTS cells) capable of generating 1-2 action potentials. Most LTS cells had non-linear current-voltage (I-V) relations and a long membrane time constant (22.5 $\pm$ 2.0 ms, SEM). The remaining 40% of PVN neurons showed no low-threshold spike (non-LTS cells), linear I-V relations and a shorter time constant (15.5 $\pm$ 2.0 ms). The LTS and non-LTS neurons had similar input resistances (230 $\pm$ 18 MΩ and 200 $\pm$ 25 MΩ), resting potentials (60 $\pm$ 2.5 mV and 63 $\pm$ 3.0 mV) and action potential amplitudes (62 $\pm$ 1.0 mV and 66 $\pm$ 1.5 mV from threshold). Following electrophysiological characterization, some cells were injected with biocytin (Horikawa and Armstrong, J. Neurosci. Meth., 25:1, 1988) and neurophysin immunohistochemistry was performed (n=7). Two of 3 injected non-LTS neurons were neurophysin-positive, suggesting they were magnocellular; 4 of 4 LTS neurons were neurophysin-negative, suggesting they were parvocellular. We suggest, therefore, that magnocellular and parvocellular neurons can be distinguished based on their intracellular electrophysiology. Supported by AFOSR 87-0361.

431.17

CONTRASTING EFFECTS OF NMDA AND NON-NMDA ANTAGONISTS ON FAST EPSPs IN NEURONS OF THE PARAVENTRICULAR NUCLEUS. J.-P. Wuarin* and F.E. Dudek Mental Retardation Res. Ctr., UCLA Sch. of Med., Los Angeles, CA 90024

Excitatory amino acids may mediate most of the fast excitatory synaptic transmission in the supraoptic nucleus (Gribkoff, V.K. and Dudek, F.E., Brain Res. 442:152, 1988). Using the paraventricular nucleus (PVN), we applied antagonists for specific amino acid receptors to determine the respective contribution of N-methyl-d-aspartate (NMDA) and non-NMDA receptor subtypes to the excitatory postsynaptic potential (EPSP) and current (EPSC). Intracellular recordings were obtained from slices of guinea-pig hypothalamus in 50 μM picrotoxin. Synaptic activation was obtained by electrical stimulation of the region dorsolateral to the fornix. The non-NMDA antagonist, 6-cyano-2,3-dihydroxy-7-nitroquinoxaline (CNQX), induced a dose-dependent decrease of the EPSP and EPSC: 1 μM had no detectable effect, 3 μM and 10 μM produced 30% and 70% decrease respectively, and 30 μM almost completely blocked the synaptic response. The NMDA-selective antagonist, D,L-2-amino-5-phosphonopentanoic acid (AP5), applied at 30 μM did not affect EPSP or EPSC amplitude or duration even when the cell was depolarized. These results suggest: 1) non-NMDA receptors mediate fast excitatory synaptic responses within the PVN and 2) although NMDA receptors may be present on PVN neurons, they seem to be less important than non-NMDA receptors in normal synaptic transmission. These data support the hypothesis that excitatory amino acids, acting primarily or exclusively on non-NMDA receptors, are the major excitatory neurotransmitter system in the hypothalamus. Supported by DAO5711, AFOSR87-0361, and the Swiss NSF.

431.18

CELL PROPERTIES AND CHOLINERGIC RESPONSES OF NEURONS FROM THE GUINEA PIG SUPRAOPTIC NUCLEUS (SON). K.R. Erickson*, O.K. Ronnekleiv and M.J. Kelly. (SPON: O. Marin) Dept. of Physiology, Oregon Health Sci Univ., Portland, OR 97201

Intracellular recordings were made from 93 SON neurons in the guinea pig to study their properties and responses to cholinergic agents. 26 (28%) were identified as vasopressinergic (VP) by their phasic firing pattern and/or by immunocytochemical staining after injection with biocytin. No significant differences were found between this group and the remaining neurons for action potential height (58.6±0.8mV), RMP (-55.8±0.7mV), firing threshold (-51.9±0.8mV), or τ (20.5 ±1.0 ms). R_{in} differed significantly (404.1 ±27.9 MΩ for VP vs. 318.3±13.9 MΩ). A time-dependent rectification (TDR) to hyperpolarizing pulses below -80 mV was seen in VP (N=9), oxytocinergic (OX,N=1), and unidentified (N=10) neurons. In 2 VP and 1 OX neuron, Cs^+ (2mM) was applied and completely blocked the TDR. 18 VP neurons were tested with Ach (N=5), nicotine (N=10), and muscarine (N=8). Ach (10-100 μM) hyperpolarized all VP cells tested. Muscarine (10 μM) hyperpolarized all but one VP cell. Although nicotine (100 μM) depolarized 8 of 10 VP cells, it did not induce phasic firing, which could be induced by the α1-adrenergic agonist methoxamine at 100 μM dose (N=7). Thus, in guinea pig VP cells, nicotinic effects are limitted to depolarization, whereas methoxamine induces long-lasting phasic activity. (Support: AHA OR Affiliate, PHS DA 05158)

431.19

RECORDING FROM IDENTIFIED ISOLATED MAGNOCELLULAR NEUROENDOCRINE CELLS (MNCs) OF THE HYPOTHALAMUS. P. Cobbett and M.L. Weiss Pharmacology & Neuroscience Program, Michigan State Univ., E. Lansing, MI 48824.

A common problem with recordings from acutely dissociated central neurons is the identification of the recorded cells. We have made recordings from acutlely dissociated MNCs which have been identified by prior labeling in vivo. Neurons were obtained from the supraoptic nucleus of the hypothalamus of adult rats by enzymic and mechanical dissociation, 1-2 days after intravenous injection of Evans Blue (EB). This dye, applied i.v., specifically labels central neuronendocrine cells (see Weiss & Cobbett, this meeting). Isolated MNCs in these preparations were identified using epifluorescence: EB excitation and emission did not affect the viability of isolated MNCs based upon the appearance of the soma after exposure to exciting wavelengths of light. Whole cell patch clamp recordings were made from identified MNCs. Resting potentials were -30mV - -63mV, and spontaneous overshooting action potentials were recorded. Under voltage clamp, action potentials were seen to be generated by a TTX-sensitive Na current. Na action potentials lasted < 10ms, but longer spontaneous action potentials (5-20s) were recorded after addition of TTX and Ba (5mM) to the external medium. Supported by NS 08125 and by a grant from the Pharmaceutical Manufacturers Association Foundation.

431.20

RESPONSES OF RAT LATERAL HYPOTHALAMIC NEURONAL ACTIVITY TO FASTIGIAL NUCREUS STIMULATION. Y. Oomura, B-I. Min* and T. Katafuchi*. Dept. of Physiology, Fac. of Med., Kyushu Univ., Fukuoka, 812 JAPAN.

The aim of this study was investigation of neuronal mechanisms underlying inputs from the fastigial nucleus (FN) to the lateral hypothalamic area (LHA). In male acesthetized rats, 295 extracellular and 82 intracellular recordings of LHA responses to electrical stimulation of the FN were examined. Contralateral FN stimulation evoked three types of responses in 48% of spontaneously firing LHA neurons: inhibition with 11 ± 6 (SD) ms latency followed by excitation (30%), excitation with 15 ± 12.5 ms latency (14%), and excitation followed by inhibition with 6 ± 4 ms latency (4%). These reponses were unaffected by transection of the inferior cerebellar peduncle. Neuronal activity was recorded intracellularly from 82 LHA neurons, of which 36 (44%) responded to FN stimulation. Of the 36 neurons, 24 showed inhibitory postsynaptic potentials (IPSPs) with a mean latency of 7.5 ± 2 ms. Of the 24 neurons, 16 were checked for change in IPSP latency with stimulus intensity, and 11 were considered to be monosynaptically connected since their latencies were constant when FN stimulation intensity was changed. The remaining 12 exhibited excitatory postsynaptic potentials (EPSPs) with a longer latency of 10.5 ± 3 ms, which indicated polysynaptic conduction. The reversal potentials of the IPSP and EPSP were estimated to be about -77 mV and -13 mV, respectively. Most glucose-sensitive neurons (78%), which were identified by their inhibition in response to electrophoretically applied glucose, were inhibited by FN stimulation, whereas only 7% of the glucose-insensitive neurons responded to such stimulation. It was concluded that LHA neurons receive inhibitory monosynaptic and excitatory polysynaptic inputs from the FN, which may contribute to hypothalamic modulation of feeding behavior.

431.21

LOCOMOTION BY THE ANESTHETIZED RAT INITIATED BY ACTIVITY OF HYPOTHALAMIC NEURONS. H.M. Sinnamon, M. Marciello*, P. Brastow*. Lab. of Neuropsychology, Wesleyan University, Middletown, CT 06457.

Two experiments determined if locomotion in the Nembutal-anesthetized rat produced by electrical stimulation of the lateral hypothalamus (LH) was mediated by fibers of passage descending from the preoptic basal forebrain or by directly activated neurons in the LH. Six rats received large unilateral lesions of the preoptic area and later were subjected to mapping of the hypothalamus for electrically-elicited locomotion at currents of 25 and 50 uA. Ipsilateral to the lesion, fewer LH locomotor sites positive at 25 uA were found but 4 of 6 rats showed some 25-uA sites and all rats had ipsilateral positive sites at 50 uA. In the hypothalamus of 8 intact rats injections of glutamate (50 mM, 50 nl) were tested in 66 sites. Injections in the LH at the level of the posterior hypothalamus nucleus produced short-latancy (<20 s) episodes of hindlimb locomotion. Descending fibers of passage contribute to, but are not essential to, the elicitation of locomotion by electrical stimulation of the LH.

431.22

EVIDENCE FOR A FUNCTIONAL AND ANATOMICAL RELATIONSHIP BETWEEN THE LATERAL SEPTUM AND THE HYPOTHALAMUS IN THE CONTROL OF FLANK MARKING BEHAVIOR IN GOLDEN HAMSTERS C.F.Ferris, L.Gold* and M.Potegal. (SPON:S.B Gagliardi) Physiology, Univ. of Mass. Med. Ctr., Worcester, MA 01655

Golden hamsters communicate by flank marking, a behavior that is controlled by vasopressin-sensitive neurons in the anterior hypothalamus (AH). The magnocellular neurosecretory system is thought to be a source of neurotransmitter for the initiation of flank marking. The anatomical and functional connections between the lateral septum and the vasopressin (VP) neurons in and around the AH were examined by: 1) tracing afferent and efferent connections following injection of HRP into the lateral septum, and 2) recording odor-induced flank marking prior to and following ibotenate lesions in the lateral septum. The greatest number of retrogradely labeled perikarya were found in the lateral hypothalamus, ventral to the fornix. Anterogradely labeled nerve terminals were primarily localized perinuclear to the VP neurons of the medial aspect of the SON. The SON, PVN and SCN were devoid of HRP-reactive nerve endings and perikarya. The injection of ibotenate into the lateral septum significantly reduced odor-induced flank marking as compared to control injections of 0.9% NaCl. These data suggest that VP neurons of the medial SON receive excitatory input from the lateral septum to facilitate flank marking behavior. (NIH Grant #NS23557)

431.23

PROJECTIONS OF THE PARAVENTRICULAR NUCLEUS OF THE HYPOTHALAMUS TO THE SEXUALLY DIMORPHIC LUMBOSACRAL REGION OF THE SPINAL CORD. C.K. Wagner and L.G. Clemens. Neuroscience Program and Dept. Zoology, Michigan State University, East Lansing, MI 48824

The paraventricular nucleus (PVN) of the hypothalamus consists of several subnuclei, some of which project to the neurohypophysis, others to brainstem and cervical-thoracic regions of the spinal cord. Lumbar regions L5-L6 of the spinal cord contain the sexually dimorphic motor nuclei, the spinal nucleus of the bulbocavernosus (SNB) and dorsolateral nucleus (DLN), which innervate perineal muscles, the bulbocavernosus and the ischiocavernosus, respectively. These motoneurons and their afferent input are androgen-dependent. This study examined projections of the PVN to the L5-L6 region of the spinal cord.

WGA-HRP (0.5-0.8ul) was injected into the region of L5-L6 aimed at the SNB and DLN and their dendritic extents, in intact male, castrated male or female rats. Following a 4 day survival time animals were perfused and HRP was visualized using the TMB method of Mesulam, (1976).

WGA-HRP labelled cell bodies were found in the parvocellular subnuclei of PVN, as well as regions of the lateral hypothalamus (LH) and the dorsal hypothalamic area (DA). These results demonstrate that the PVN projects to lumbar levels of the spinal cord that are sexually dimorphic and androgen-dependent. This suggests that PVN may modulate the activity of these motoneurons.

431.24

EFFERENTS OF THE SEXUALLY DIMORPHIC AREA OF THE GERBIL HYPOTHALAMUS Patricia D. Finn*, Geert J. DeVries, and Pauline Yahr. Dept of Psychobiology, Univ of California, Irvine, CA 92717.

The sexually dimorphic area (SDA) of the gerbil hypothalamus (hyp) accumulates gonadal steroids and is implicated in the hormonal control of male sexual behavior and scent marking. To identify pathways for these behaviors, SDA efferents were traced in both sexes by injecting Phaseolus vulgaris leucoagglutinin (PHA-L) into the medial or lateral SDA (mSDA; lSDA) and locating PHA-L-filled fibers. SDA efferents were verified by injecting putative terminal fields with the retrograde tracer Fluoro-Gold.

The ventrolateral (vl) septum, medial bed nucleus (n) of the stria terminalis, medial amygdala (MeA), substantia innominata, anterior hyp, periventricular n, arcuate n, vl ventromedial n (vlVMN) and ventral premammillary n receive more mSDA than lSDA. The opposite is true for the lateral hyp and retrorubral field. Both mSDA and lSDA project to the posterior hyp, lateral and supramammillary n, paraventricular thalamic n, reuniens, central gray and most raphe n. The vlVMN and posterodorsal MeA were most heavily labeled when the PHA-L injection overlapped the SDA pars compacta, a cell group normally found only in males. Areas yet to be verified include rostral preoptic areas, dorsomedial n, supraoptic n, paraventricular hyp n, zona incerta, ventral tegmentum, locus coeruleus, laterodorsal n and solitary n. (Support: MH-14599 and MH-26481)

431.25

EFFECT OF OVARIAN CONDITION ON SEXUALLY DIMORPHIC BRAIN AREAS IN A WHIPTAIL LIZARD SPECIES. J. Wade*[1] and D. Crews.[1,2] Departments of [1] Psychology and [2] Zoology, and the Institute of Reproductive Biology, University of Texas, Austin, TX 78712.

Both the anterior hypothalamus-preoptic area (AH-POA) and the ventromedial hypothalamus (VMH) are sexually dimorphic in the whiptail lizard, *Cnemidophorus inornatus*. The AH-POA is significantly larger in males, while the VMH is significantly larger in females (D. Crews, J. Wade and W. Wilczynski, unpublished). To determine whether these brain areas change in volume with the breeding season, the AH-POA and VMH of females were measured under three conditions: reproductively active (during the breeding season); reproductively inactive (during a simulation of winter hibernation conditions); and ovariectomized. Measurements were taken on a Zeiss Videoplan by tracing the perimeter of the AH-POA and VMH on one side of the brain in every fifth section. The computer generated areas of these brain regions. The volumes were calculated from the areas based on the distance between the sections measured (125μ). An estimate of overall brain size was calculated by tracing the perimeter of one-half the brain at the most anterior section of the POA and again 30 sections later (approximately midway through the VMH). Volume was calculated from these areas as if they were the ends of a cone frustum. Relative to estimates of overall brain volume, there was no significant difference among the volumes of the AH-POA in the three groups (ANOVA on transformed ratios: $F(2,23)=2.19$, $p=.13$). However, the VMH of reproductively active animals was significantly larger than those of animals in the other two groups ($F(2,23)=5.78$, $p=.01$). These data suggest that the volume of the VMH may change seasonally, perhaps under the influence of ovarian steroids.

This work supported by NIMH grant 41770 and Research Scientist Award 00135.

431.26

VERVET MONKEYS HAVE SEXUALLY DIMORPHIC, ESTROGEN-SENSITIVE HYPOTHALAMIC NEURAL MEMBRANES. F. Naftolin, C. Leranth, D.E. Redmond, Jr.+ and L-M Garcia-Segura*. (Spon: J. DeFelipe) Dept of Obs and Gyn and Psych+, Yale Med Sch, New Haven, CT and Inst Cajal*, Madrid, Spain.

Many sexually dimorphic characteristics of primates have neural bases. In rats, a similar picture exists in the hypothalamic nuclei which control reproductive function, and is associated with an estrogen-sensitive sexual dimorphism in the numerical density of intramembranous protein particles (IMP), (J Ster Biochem 30:195, 1988.) We studied the hypothalamic infundibular nucleus of mature vervet monkeys to assess whether similar findings are present in primates. EXPERIMENTAL: Monkeys were lightly anesthetized and perfusion-fixed using a physiologic saline wash followed by 1% glutaraldehyde - 1% paraformaldehyde. The infundibular nucleus was microdissected for freeze-fracture analysis. Neurons were recognized by morphologic characteristics of their cytoplasm. Three animals were studied in each group; for each the E- and P-faces of at least 10 μM^2 of neuronal membrane were evaluated. There was a clear difference in numerical density of P-face IMP between normal males and females (869 $\pm$ 34 SEM vs 1432 $\pm$ 31 IMP/μM^2, respectively; $p < 0.001$). The E-face, with a lower density of IMP, also showed a significant sex difference. While ovariectomy did not effect the density of IMP (1427 $\pm$ 41), ovariectomy plus estradiol treatment 48 hours before perfusion dramatically lowered the numerical density of IMP to that seen in males (812 $\pm$ 29). CONCLUSIONS: Vervet monkeys have sexually dimorphic hypothalamic neural membranes, and estrogen treatment abolishes the sex difference. These findings, including the actual range of IMP/μM^2 and the effect of estradiol, are the same as in the rat. They raise the possibility that other estrogen-sensitive characteristics of hypothalamic neurons, such as estrogen-induced synaptogenesis, synaptolysis and cyclic synaptic remodelling will also be found (J Ster Biochem, ibid) and may play important roles in estrogen-dependent states of health and disease in monkeys and other primates, including humans.

(Supported by NIH-HD13587 and CSIC, Spain.)

431.27

MECHANISMS OF THE INITIAL PHASE OF FEVER IN THE VSA AND MAHPOA OF RATS AND RABBITS. G.E. Resch*, C.W. Simpson*, W.D. Ruwe., and J.Clarke* Sch. Basic Life Sci., Univ. Missouri-KC, Kansas City, MO 64108 and Dept. Physiol. Biophys., Univ. Arkansas Col. Med. Sci., Little Rock, AR 72205.

Mechanisms underlying the initiation of pyrogen induced fever were studied at two brain heat-gain sites in rats and rabbits. Three questions were addressed. The first question was to identify brain sites at which microinjections of bacterial or endogenous pyrogens evoked a classically defined fever. The second question considered whether blockade of neuronal activity at these sites would modify the initial and subsequent phases of a fever evoked by bacterial pyrogens or endogenous leucotrienes. The third question tested the relationship of PGE_2 in the mediation of the fever response. E. coli, PGE2, alpha-interferon, and IL-1B elicited fever from the ventral septal area (VSA) and MAHPOA of rats and rabbits. The fever response consisted of an early peak within 1 hr. and a 2nd peak within 3-6 hrs. The PGE2 antagonist, SC19220, or atropine blocked fever from IL-1B in the MAHPOA. Blockade of the initial phase of the fever suggests PGE2 and Ach may mediate the initial events. The data demonstrate two brain sites, the VSA and the MAHPOA, in two species which are similar in their response to hyperthermic agonists, also responded to bacterial pyrogens and leucotrienes. Specific receptor antagonists at the MAHPOA site block the initial phase of a fever following microinjection of IL-1B. (Supported in part by USAFOSR-87-0297 and by NIH-NS26045).

431.28

AGE-RELATED CHANGES IN THE THERMOREGULATORY RESPONSES TO CYTOKINES IN THE NEW ZEALAND WHITE RABBIT. J. Clarke* and W.D. Ruwe (SPON: L. Plunkett). Dept. Physiol. Biophys., Univ. Arkansas for Med. Sci., Little Rock, AR 72205.

The ability of the male New Zealand white rabbit to develop fever in response to central administration of pyrogens is age dependent. Experiments were conducted in both young and old rabbits to characterize the thermal response to central injections of cytokines acting as endogenous pyrogens (EPs). Guide tubes were implanted bilaterally above the anterior hypothalamic, preoptic area (AH/POA) in young (<1 yr) and old (>3 yrs) rabbits. Bacterial pyrogen (E. coli), EPs (IL-1, α-IFN, and IL-6), and thermoregulatory mediators (PGE_2, NE, and 5-HT) were injected into the AH/POA (bilaterally, 0.5-1.0μl) and T_{core} and T_{skin} monitored for 6 hours following injection. E. coli elicited a fever in old animals that was significantly less than that evoked in the young. Fevers caused by IL-1 and α-IFN also were reduced in the older group. However, the fever evoked by IL-6 in the older rabbits initially was diminished but at 3-6 hrs was significantly greater. Thermoregulatory responses to PGE_2 and NE also were age-related. These results suggest that as the animal ages: (1) fevers evoked by pyrogens are consistently reduced; (2) responses to mediators of thermoregulation may be diminished likewise; and (3) fevers elicited by the EPs may be altered differentially. [Supported in part by NIH #NS26045 to W.D.R.]

431.29

INTRACELLULAR ANALYSIS OF INHERENT AND SYNAPTIC ACTIVITY IN HYPOTHALAMIC THERMOSENSITIVE NEURONS M.C. Curras and J.A. Boulant. Physiology Dept., Ohio State University, Columbus, OH 43210

To understand neuronal thermosensitivity, intracellular activity was recorded in rostral tissue slices of rat hypothalamus. Neurons had mean resting membrane potentials of -55 mV, and 30 neurons were classified according to thermosensitivity. Even though synaptic activity is present, warm sensitive neurons display inherent thermosensitivity due to a temperature dependent pacemaker potential. Neuronal cold sensitivity, however, is primarily attributed to temperature dependent changes in excitatory and inhibitory synaptic input. Cold sensitive neurons also show increased input resistance during cooling, which could enhance the effectiveness of the excitatory synaptic input. Some temperature insensitive neurons can display a "functional plasticity" in which transient periods of warm and cold sensitivity occur. This supports a previous study suggesting that temperature insensitive neurons possess a voltage dependent (thermosensitive) pacemaker current that is usually suppressed by an electrogenic Na-K pump. (Supported by NIH and the Bremer Foundation.)

DRUGS OF ABUSE: DOPAMINE MECHANISMS

432.1

DOPAMINE RECEPTOR REGULATION BY SUBCHRONIC COCAINE AND METHAMPHETAMINE TREATMENTS. A.B. Kelly*, S.S. Overman*, H.H. Osterburg*, G.M. Pasinetti, M.M. Myers*, B. Knapp*, C.E. Finch & D.G. Morgan. Dept. of Biological Sciences and Andrus Gerontology Center, Univ. of Southern California, Los Angeles, CA, 90089-0191.

Young male Fischer 344 rats were injected i.p. with 10 mg/kg methamphetamine HCl, 20 mg/kg cocaine HCl, or saline vehicle twice daily for 10 d. Striata from these animals (n=8 per group) were dissected 14 and 60 days after the last day of the drug treatments and frozen, and the hindbrain was immersion fixed in paraformaldehyde. Striatal dopamine receptors were measured using [3H]SCH-23390 (D-1 receptors) or [3H]spiperone (D-2 receptors). Striatal dopamine and DOPAC were measured by HPLC. For each rat, twelve 10 um paraffin sections of the substantia nigra were immunostained for tyrosine hydroxylase (TH) or GFAP. At the 14 day survival time, methamphetamine produced a 20% reduction in the Bmax of both D-1 and D-2 receptors, with no change in Kd. No changes were observed in the concentrations of dopamine or DOPAC, the number of nucleated TH immunopositive nigral neurons, or the number of GFAP immunopositive nigral astrocytes. Cocaine had no effects on any of these parameters. At the 60 day survival time, cocaine treatment produced a 20% increase in the Bmax of D-1 and D-2 receptors, with no change in Kd. Methamphetamine produced a 20% reduction in the Bmax of D-1 and D-2 receptors, but this effect was not statistically significant at the $P < 0.05$ criterion. Neither treatment influenced the number of TH immunopositive neurons, nor GFAP immunopositive astrocytes in the substantia nigra. Hence, methamphetamine can produce a decline in dopamine receptors without nigral neuron atrophy. Cocaine appears to produce a slowly developing increase in striatal dopamine receptors. Supported by the USC B.R.S.G. and the Anna Greenwall Award from the AFAR.

432.2

COMPARISON OF [^{3}H]GBR 12935 AND [^{3}H]COCAINE BINDING SITES IN MONKEY BRAIN. M.A.Fahey*, D.R. Canfield*, R.D. Spealman*, B.K. Madras (SPON: R. Milius). Harvard Medical School, New England Regional Primate Research Center, Southborough, MA 01772.

Both [^{3}H]GBR 12935 and [^{3}H]cocaine label elements of the dopamine transporter in striatal tissue. We have directly compared the binding of drugs to sites labeled with either [^{3}H]cocaine or [^{3}H]GBR 12935 in caudate-putamen membranes of monkeys (M. fascicularis). Assays were performed at 4°C in Tris-HCl (50 mM) buffer containing NaCl (120 mM). The sites labeled with [^{3}H]GBR 12935 and [^{3}H]cocaine were similar with respect to density, Na^+ sensitivity and rank order of displacement by neurotransmitters (dopamine > norepinephrine > serotonin). However, cocaine congeners including (-)-cocaine, CFT (WIN 35,428), WIN 35,065-2, N-allyl CFNT, and (+)-cocaine did not displace [^{3}H]GBR 12935 fully, and their IC_{50} values at [^{3}H]GBR 12935 binding sites were 5-20 times higher than their IC_{50} values at [^{3}H]cocaine receptors. Conversely, structurally unrelated monoamine uptake inhibitors including Lu 19-005, GBR 12909, and mazindol did not displace [^{3}H]cocaine fully, and their IC_{50} values were comparable at both sites. The results show that [^{3}H]GBR 12935 and [^{3}H]cocaine label related, though probably not identical, elements of the dopamine transporter. Supported by DA00499, DA00088, RR00168.

432.3

CELLULAR EFFECTS OF ACUTE COCAINE IN THE RAT NEOSTRIATUM. K. Nantwi and E.P. Schoener,* Dept. of Pharmacology, Wayne State University School of Medicine, Detroit, MI 48201.

Euphoria and reinforcement that occur with cocaine have been attributed to dopamine-mediated neurotransmission. The present study examined electrophysiologic manifestations and dopaminergic mechanisms of systemically administered cocaine in the rat neostriatum.

Cocaine was found to modify neuronal firing rate over a dose range of 0.1-1.0 mg/Kg, while administration of drug vehicle did not result in any significant change of neuron discharge. Of thirty-two (32) units tested at 0.25 mg/Kg cocaine, 53% were facilitated while 47% were inhibited. At higher doses of cocaine, a larger proportion of units exhibited depression after drug exposure. For example, 68% had reduced activity and 32% enhanced discharge after 0.5 mg/Kg. Similar changes in neuronal activity were produced by acute infusion of L-DOPA (0.01-1.0 mg/Kg) following pretreatment with carbidopa (0.1 mg/Kg). In control experiments, carbidopa itself did not modify neuronal activity. Cocaine effects were altered by pretreatment with dopamine antagonists. Experiments with the selective DA-1 antagonist SCH 23390 reversed, blocked, or attenuated the effect of cocaine in a dose-related manner. These data indicate that the changes in neuronal activity after acute cocaine exposure may be mediated by selective dopaminergic mechanism(s).

432.4

EFFECTS OF NON-COMPETITIVE NMDA ANTAGONISTS ON EXTRACELLULAR DA LEVELS IN THE STRIATUM AS AIMED WITH MICRODIALYSIS PROCEDURE. A. Mele*, K.M. Wozniak*, J.A. Monn, A. Thurkauf, K.C. Rice and A. Pert. (Spon. J.H. McDonough, Jr.) BPB/NIMH, LCS/NIAAA and IN/NIDDK, Bethesda, MD 20892.

Considerable evidence suggests important effects of noncompetitive NMDA antagonists like phencyclidine on dopaminergic function. The effects of a number of non-competitive NMDA antagonists on alteration of striatal extracellular DA were evaluated and compared to their ability to induce rotational behavior in rats lesioned with 6-OHDA unilaterally in the medial forebrain bundle. In the microdialysis experiment systemic injections of MK 801, SKF 10,047, dexoxadrol and levoxadrol even at high doses, did not produce any effects on extracellular DA concentration, whereas PCP(10 mg/kg) and TCP (25 mg/kg) increased levels significantly. The rank order of potency in the rotational study, however was found to be: MK> PCP> dexoxadrol> SKF. Since all drugs tested produced profound effects on rotational behavior but only TCP and PCP enhanced DA levels in the striatum, it appears that noncompetitive NMDA antagonists act on the intact nigrostriatal system through DA independent mechanisms to produce rotational behavior.

432.5

INDUCTION OF C-FOS BY DIRECT AND INDIRECT DOPAMINE AGONISTS S.T. Young*[1], L.J. Porrino[2], and M.J. Iadarola[3] (SPON: G. Dienel). [1]LCM, NIMH and HHMI; [2]CNB, NINDS; and [3]NAB, NIDR, Bethesda MD 20892

The proto-oncogene c-fos produces a nuclear phosphoprotein Fos which is thought to act as a genetic transactivator regulating gene expression. Reports of alterations in neuropeptide gene regulation following dopamine agonist treatment prompted us to assess whether c-fos induction was a component of dopaminergic stimulation. Fos expression was examined immunohistochemically in 30 um sections of rat forebrain. Rats treated with the indirect dopamine agonist, cocaine (30 mg/kg, IP), showed a significant increase in Fos expression in both the number of cells and intensity of nuclear immunoreaction in the caudate/putamen relative to saline controls. The highest density of immunoreactive neurons was in the dorsomedial and dorsocentral aspects of the caudate. This effect was apparent 1 hour after treatment, maximal at 2 hours, and not different from control levels 48 hours after treatment. Increases in Fos expression were also noted in the olfactory tubercle, although levels of Fos in the nucleus accumbens and primary olfactory cortex were not significantly different from saline controls in most cases. Animals administered the direct agonist, apomorphine (SC), also displayed a similar increase in Fos staining in the caudate/putamen (again mainly dorsomedial/dorsocentral) relative to saline controls. At low doses (0.1 mg/kg), the difference from control was small compared to the increase seen at 1 mg/kg and 5 mg/kg. These results demonstrate a role for dopamine in the induction of c-fos expression, and, by extension, suggest a change in expression of one or more striatal genes. They suggest that, in addition to acute changes in neuronal firing rate, activation of dopamine systems in the CNS may have other, longer-lasting effects on neuronal function via transcriptional modulation of certain target genes in activated cells.

432.6

METHAMPHETAMINE NEUROTOXICITY: RESIDUAL CAPACITY OF NIGROSTRIATAL DOPAMINE NEURONS STUDIED WITH MICRODIALYSIS. J. Yew*, P. Paulson*, D. M. Camp*, L. Ruddock*, L. Levy* and T.E. Robinson. (SPON. M.Alpern) Dept. Psychology, Neuroscience Program, The University of Michigan, Ann Arbor, MI 48109.

Repeated exposure to high doses of methamphetamine (MA) greatly depletes cerebral dopamine (DA) and serotonin (5-HT), but does not produce gross deficits in spontaneous behavior. We hypothesized that sparing of function may occur, at least in part, because the remaining DA neurons are able to maintain relatively high synaptic concentrations of DA. To test this idea the concentration of DA, dihydroxyphenylacetic acid (DOPAC), homovanillic acid (HVA) and 5-hydroxyindoleacetic acid (5-HIAA) was measured in striatal extracellular fluid (ECF) by microdialysis in freely moving rats. The animals were pretreated one week earlier with either saline or MA (15 mg/kg/6 hr x 6). MA pretreatment produced a large decrease (50-90%) in the basal ECF concentration of DOPAC, HVA and 5-HIAA, presumably reflecting the loss of DA and 5-HT terminals in striatum. In contrast, the basal ECF concentration of DA was not changed significantly by MA pretreatment. Furthermore, both saline and MA-pretreated rats showed a large increase in motor activity and the ECF concentration of DA in response to a challenge injection of 1.5 mg/kg of *d*-amphetamine. It is suggested that residual DA neurons can compensate for the depletion of striatal DA produced by highly neurotoxic doses of MA. Whether residual 5-HT neurons have a similar compensatory capacity is currently under investigation.

432.7

PERSISTENT CHANGES IN BRAIN CATECHOLAMINE AND MONOAMINE LEVELS AFTER REPEATED COCAINE INJECTIONS. J. Peris and R. Dawson. Dept. Pharmacodynamics, Univ. Florida J. H. Miller Health Ctr., Gainesville, FL 32610.

Repeated administration of cocaine results in long-lasting sensitization to the initial locomotor activating and stereotypic effects of cocaine. Since cocaine inhibits dopamine (DA), norepinephrine (NE) and serotonin (5-HT) reuptake into neuronal endings, changes in one or all of these neurotransmitter systems may cause sensitization. We measured whether any changes in DA, NE, 5-HT or their metabolites are as long-lasting as the behavioral changes. Male Sprague-Dawley rats were injected with either saline or cocaine (10 mg/kg, i.p.; n = 6) daily for eight days and stereotypy and locomotor activity were measured after the first, second, and eighth injections. After a seven day withdrawal period from cocaine, rats were tested with either saline or cocaine and then seven days later were sacrificed for brain dissection. Levels of DA, NE, 5-HT, DOPAC, HVA, and 5-HIAA were measured in nucleus accumbens, striatum, frontal cortex, olfactory bulb, olfactory tubercle and ventromedial mesencephalon using reverse phase HPLC with electrochemical detection. Concentrations of 5-HT were significantly decreased in olfactory tubercle of cocaine-treated animals (538±38) compared to that of saline-treated animals (669±60) and 5-HIAA/5-HT ratios were increased in the cocaine-treated animals (0.64±.05 vs. 0.50±.05). There were no differences in catecholamine or monoamine levels due to cocaine treatment in any other brain region tested. These data suggest that some of the persistent effects of repeated cocaine administration on behavior may be mediated by serotonergic rather than catecholaminergic mechanisms.

432.8

METABOLIC MAPPING OF THE ACUTE AND CHRONIC EFFECTS OF METHAMPHETAMINE IN THE RAT. F.E. Pontieri*, Y.A. Ryan*^, M.S. Kleven~, L.S. Seiden~ and L.J. Porrino^ (SPON: C Kennedy). NIMH, Bethesda, MD 20892, ^NINDS, Bethesda, MD 20892, ~University of Chicago, Chicago, IL 60637.

Using the quantitative 2-[^{14}C]-deoxyglucose autoradiographic method (2-DG), we compared the acute and chronic effects of methamphetamine (MA) administration on local cerebral glucose utilization (LCGU) in freely-moving rats. Four groups of animals received 0.0, 0.5, 1, 2.5 mg/kg i.v. MA, respectively, 10 minutes before the 2-DG procedure. The acute i.v. administration of MA resulted in dose-dependent alterations of LCGU in the extrapyramidal system (e.g. caudate, globus pallidus, entopeduncolar nucleus, substantia nigra, subthalamus and lateral habenula). Cerebral metabolic rate was also increased in portions of the mesocorticolimbic system (e.g. nucleus accumbens, VTA and septum). This pattern of alterations is similar to those seen following the administration of other psychostimulants (cocaine and amphetamine). In addition, LCGU was significantly increased in structures related to the visual system, suggesting a possible neural substrate for visual hallucinations that accompany MA abuse in humans. A second series of rats was treated with 50mg/kg of MA or vehicle s.c. twice daily for 4 days, a regimen known to produce long-lasting depletion of dopamine and serotonin. LCGU was measured 14 days after treatment, when monoamine depletion is well-established. In contrast to rats treated acutely, rats treated chronically had depressed rates of LCGU, particularly in the extrapyramidal system. These data suggest that alterations in functional activity following chronic MA treatment are present in regions other than just those in which monoamine depletion is known to occur.

432.9

THE EFFECTS OF SCH 23390 ON REWARDING BRAIN STIMULATION: EVIDENCE FOR PARTIAL D1 MEDIATION OF THE THRESHOLD LOWERING EFFECTS OF MDMA. M. Bird and C. Kornetsky. Boston University School of Medicine, Boston, MA 02118.

Previous studies from our laboratory have provided pharmacologic evidence that the increased sensitivity to rewarding brain stimulation caused by abuse substances is mediated primarily by dopaminergic processes. Using this model of drug induced euphoria, we have recently shown that the reward threshold lowering caused by racemic 3,4-methylenedioxymethamphetamine (MDMA) is blocked by D2 but not by 5-HT2 receptor antagonism (Abst. Soc. Neurosci. 13:1323, 1987). To further examine the role of DA receptor subtypes in this reward system we studied the effect of D1 blockade on MDMA's enhancement of rewarding brain stimulation using the selective D1 antagonist SCH 23390. Four male albino F-344 rats were implanted with bipolar electrodes into the medial forebrain bundle and the effects of SCH 23390 alone and in combination with a maximally reinforcing dose of MDMA were determined. In each animal reward thresholds were systematically raised by SCH 23390 and MDMA's threshold lowering action was antagonized. This suggests that both D1 and D2 receptors are necessary but not sufficient substrates for MDMA's pharmacologic activation of central reward pathways.

(Supported in part by NIDA grant DA 02326 and Research Scientist Award DA00099 to CK).

432.10

^{3}H-WIN 35,065-2 (^{3}H-WIN) BINDING IS SIMILAR IN VARIOUS BRAIN REGIONS OF THE RAT. J.W. Boja and M.J. Kuhar. Neuroscience Branch, NIDA Addiction Research Center, Baltimore, MD 21224.

Present evidence suggests that cocaine binds to the dopamine transporter at both low and high affinity sites. In addition, previous studies have shown that cocaine binding in Tris buffer is sodium dependent at the cocaine binding site. Since dopamine terminals in the striatum (STR) arise from the nigro-striatal neurons while the dopamine terminals in the nucleus accumbens (NA) arise from mesolimbic dopamine neurons, other laboratories suggested that cocaine can interact differently at these two areas. This is of interest since the NA has been implicated as one of the sites of cocaine's reinforcing properties, while the STR has not. ^{3}H-WIN, a potent cocaine analog, was used to assess binding to the dopamine transporter in the STR and NA. Binding of ^{3}H-WIN in Tris buffer (50 mM Tris, 120 mM Na^+, 5 mM K^+ pH 7.4) was Na^+ dependent in both the NA and the STR at both the low and high affinity cocaine binding sites. (-)Cocaine inhibited ^{3}H-WIN binding in a dose dependent manner at both the NA (IC_{50} = 1.96 µM) and the STR (IC_{50} = 1.86 µM). These data suggest cocaine binding is similar in the NA and the STR.

432.11

^{3}H-WIN 35,065-2(^{3}H-WIN): A LIGAND FOR COCAINE RECEPTORS IN RAT STRIATUM. M.C. Ritz, J.W. Boja, F.I. Carroll*, A.H. Lewin* and M.J. Kuhar. Neuroscience Branch, Addiction Research Ctr., Baltimore, MD 21224 and Research Triangle Institute, Research Triangle Park, NC 27709.

Several radiolabeled compounds have been used to identify behaviorally relevant cocaine receptors in brain. WIN is a compound structurally similar to cocaine, more potent in behavior and binding assays, and relatively more resistent to metabolic breakdown. Thus, ^{3}H-WIN was synthesized and examined in receptor binding assays. Rat striatal tissue was homogenized in 50 mM Tris buffer containing 120 mM NaCl and 5 mM KCl. Incubations were carried out at 0-2°C for 60 min. ^{3}H-WIN binding was saturable and revealed two binding sites (K_{D1}=5 nM, B_{max1}=4.5 fm/mg tissue; K_{D2}=150 nM, B_{max2}=70 fm/mg tissue). The binding was stereospecific, sodium dependent and relatively insensitive to increases in incubation temperature. Compounds potent at the cocaine binding site and compounds potent in inhibiting dopamine uptake were also potent inhibitors of WIN binding (mazindol, GBR12909, nomifensine and (-)cocaine). Inhibitors of norepinephrine uptake such as desipramine and the serotonin uptake inhibitor citalopram were extremely weak inhibitors of ^{3}H-WIN binding. Thus, ^{3}H-WIN is a suitable ligand for cocaine receptors associated with the dopamine transporter and may have some advantages over other existing ligands.

432.12

SELF-ADMINISTRATION OF BRAIN-STIMULATION: A NOVEL MODEL OF DRUG SELF-ADMINISTRATION. K.B.J. FRANKLIN AND M. LEPORE*., Dept. of Psychology, McGill University, P.Q. Canada, H3A-1B1.

Intracranial self-stimulation (ICSS) has long been considered a model for the hedonic effects of drugs of abuse. However, direct comparisons between ICSS and drug self-administration has seldom been possible since the characteristics of responding for 150-500 ms trains of 0.1 ms pulses at 100 Hz are very different from those observed during self-administration of a drug with complicated absorption, distribution, and excretion kinetics. We have trained rats to self-administer long trains of brain stimulation which are frequency-modulated to mimick the pharmacokinetic characteristics of stimulant drugs. There are striking similarities between this novel form of ICSS and drug self-administration. Rats often "load up" at the beginning of a session. Increasing the "dose" administered or increasing the half-life of stimulation produces corresponding decreases in the rate of ICSS. Furthermore, low doses of the dopamine antagonists SCH23390, pimozide, and alpha-flupenthixol, increase the "dose" of stimulation the animal self-administers. Thus, differences observed between conventional ICSS and drug self-administration behaviors may be due to the parameters of brain-stimulation. This novel ICSS paradigm may be a useful model for studying the role of pharmacokinetics in drug self-administration.

432.13

PSYCHOACTIVE AMINES ACT BY A WEAK BASE MECHANISM IN CHROMAFFIN GRANULES AND CULTURED VENTRAL TEGMENTAL AREA (VTA) DOPAMINE CELLS. David Sulzer* & Stephen Rayport (SPON: Eric Holtzman). Dept. Psychiatry and Ctr. Neurobiol. & Behav., Columbia Univ. and New York State Psychiatric Inst., New York, NY 10032.

A number of psychoactive drugs reduce cellular uptake of catecholamines at lower concentrations and induce release at higher concentrations. We propose the novel hypothesis that these phenomena result from such drugs acting as weak bases which reduce the pH gradient across the storage granule membrane. We tested this idea in isolated adrenal chromaffin granules and cultured dopamine neurons.

Bovine adrenal chromaffin granules are incubated with the weak base vital dye acridine orange (AO) and the emission at 526 nm, which is pH sensitive, is recorded. The granules acidify with addition of ATP and valinomycin, resulting in quenching of fluorescence. The resulting pH gradient is rapidly abolished by the weak base ammonium chloride or the protonophore FCCP. Strikingly, the pH gradient is also reduced by the psychoactive amines cocaine, amphetamine, phencyclidine, tyramine, fenfluramine, ephedrine, and imipramine. These drugs act at low concentrations: 50% alkalinization is seen after incubation with 30 µM cocaine *vs.* 4.5 mM dopamine, or 1 mM ammonium chloride, a "classic" weak base. Moreover, the drugs generally act rapidly: for 30 µM cocaine τ < 1 min, whereas for 4.5 mM dopamine τ > 5 min.

In purified rat VTA dopamine neurons in culture (Rayport, Sulzer, and Batson, *this volume*), AO and neutral red (also a weak base vital dye) stain intracellular acidic compartments. Often, staining is seen in sites that correspond to the EM-localization of catecholamine-containing vesicles revealed by 5-hydroxy-dopamine uptake. Staining both at presumed vesicle sites and in lysosomes can be abolished by incubation with cocaine, amphetamine, phencyclidine, ammonium chloride, or FCCP.

433.1

NEURAL CELL-LINE TRANSPLANTATION IN THE RAT. V.W. Henderson, M. Cunningham,* N. Valtz,* R.D.G. McKay. Depts. of Neurology (Div. of Behavioral Neuroscience & Aging) and Gerontology, University of Southern Calif., Los Angeles, CA; and Depts. of Brain & Cognitive Sciences and Biology, Mass. Institute of Technology, Cambridge, MA

The HT4 and ST15A neural precursor cell lines were previously derived from rat embryonic hippocampus and neonatal cerebellum, respectively, using a temperature-sensitive conditional oncogene. In tissue culture at 33°C, HT4 and ST15A are rapidly dividing clonal cell lines; at 39°C in vitro they cease division and assume differentiated characteristics of neurons or glia.

HT4 and ST15A were labeled in vitro through incorporation of ^{3}H-thymidine or bromodeoxyuridine (BrdU). Cell suspensions were implanted into dorsal hippocampus or cerebellum of anesthetized neonatal Sprague-Dawley rats, and animals were sacrificed at intervals. In cryostat sections of fixed tissues, transplanted cells were readily identified by autoradiography (^{3}H-thymidine), immunohistochemistry (BrdU), or X-gal histochemistry for the lacZ gene product. In some animals, implanted HT4 cells assumed a position proximate to the hippocampal alveus; prominent labeling within the cerebellum granular layer occured in some ST15A-transplanted rats. There was no tumor formation. Findings confirm the feasibility of neural cell-line transplants and suggest new treatment strategies for human neurodegenerative disorders.

433.2

LONG-TERM FUNCTION OF GENETICALLY MODIFIED, NGF-SECRETING FIBROBLASTS GRAFTED INTO RATS WITH FIMBRIA-FORNIX LESIONS. M.B. Rosenberg, M.H. Tuszynski, R.C. Hayes, K. Yoshida,* D.M. Armstrong, T. Friedmann* and F.H. Gage. Depts. Pediatrics and Neurosciences, Univ. Calif. San Diego, La Jolla, CA 92093.

We have previously shown that cultured rat fibroblasts, genetically modified to secrete NGF and then grafted to the cavity formed in creating a fimbria-fornix lesion, prevent retrograde cholinergic degeneration two weeks after transplantation (Science 242:1575, 1988). The present studies were undertaken to evaluate long-term graft survival and effects on cholinergic neurons. Fibroblasts of the line Rat1, derived from embryonic Fischer rats, were infected with a retrovirus vector expressing mouse NGF cDNA and the bacterial gene for neomycin resistance. The clone with the highest NGF production, which secreted NGF at 770 pg/hr/10^{5} cells, was grafted into Fischer and Sprague-Dawley rats with unilateral aspirative fimbria-fornix lesions. Control animals received uninfected fibroblasts. Animals were sacrificed after 2 or 8 weeks, and the brains were examined for cholinergic neuron survival in the medial septal area by immunohistochemistry for choline acetyltransferase and NGF receptors. At 2 weeks there was a substantial savings of cholinergic neurons in the animals that had received NGF-secreting grafts but not in animals with control grafts. At 8 weeks, some animals still exhibited cholinergic survival. In situ hybridization with a riboprobe complementary to NGF mRNA indicated transcripts were present at 2 and 8 weeks. Immunohistochemical labelling for parvalbumin, a GABAergic neuron marker, showed that the effects of the NGF-secreting fibroblasts were apparently specific for cholinergic neurons. These studies indicate that genetically modified fibroblasts can exhibit survival and transgene expression for at least 2 months following intracerebral grafting. Additional experiments are underway to examine longer-term graft survival and function.

433.3

DEVELOPMENTAL TIME COURSE OF GRAFTS OF FETAL HIPPOCAMPUS PLACED INTO ASPIRATION LESION SITES IN ADULT RATS. D.J. Paul, R.H. Baisden, and M. L. Woodruff. Dept. of Anatomy, Quillen-Dishner Col. of Med., East Tennessee State Univ., Johnson City, TN 37614.

The mature structure of fetal hippocampal transplants associated with behavioral recovery (Woodruff et al. Exp. Neurol. 102, 130-143, 1988) differs from that of the normal hippocampus. To begin to understand the mature structure of the transplants we studied the growth of E16 fetal hippocampal transplants. In some rats the volume of the transplant was estimated 1,3,6,9,15,30 and 45 days posttransplantation. The volume was less than that transplanted until day 9 although the transplanted tissue had the appearance of healthy neuroepithelium but increased rapidly from Day 9 to Day 30. Other rats were implanted and given injections of tritiated thymidine either immediately or 12 hrs later, or 1,2,3,5,6,8,9,15 or 30 days posttransplantation. Fortyfive days following transplantation the brains were autoradiographically processed. Labeled neurons were found in the grafts through injection day 9, indicating that the rapid growth of the transplants after day 9 was due to development of neuropil. Labeled granule cells could also be observed in the host dentate gyrus several mm from the graft. These may have migrated from the transplant or be host cells induced into mitosis by the graft (e.g. Lundberg & Mollgard, Neurosci. Lett. 13, 265-270, 1979). (Supported by NIH grant ES04070-03 to MLW)

433.4

EFFECTS OF SCIATIC NERVE TRANSPLANTS ON MEDIAL SEPTAL CHOLINERGIC CELLS AFTER FORNIX/FIMBRIA LESION. D. J. Messersmith and L. F. Kromer. Dept. of Anatomy and Cell Biology, Georgetown University, Washington, DC 20007

Trophic and tropic effects of peripheral nerve transplants in the CNS were examined utilizing the septo-hippocampal pathway in adult Sprague-Dawley rats. Following a bilateral fornix/fimbria lesion, a segment of sciatic nerve was inserted on each side of the brain between the septum and dorsal hippocampus. After either 2 or 8 weeks post-transplantation, 6 animals in each experimental and control (lesion only) group were perfused. Brains were sectioned at 25u and stained for choline acetyltransferase (ChAT) or acetylcholinesterase (AChE). The number of ChAT(+) cells in the medial septum was counted in twelve sections rostral to the postcommissural fornix.

Two weeks post-transplantation there was a statistically significant increase ($p<.05$) in the number of ChAT(+) cells in the septum of animals receiving the transplant vs animals with lesions only [Mean ± SEM: 2003 ± 258 (transplant) vs 1011 ± 162 (control)]. However, after eight weeks there was no longer a statistical difference between transplant and eight week lesion control [1593 ± 117 (transplant) vs 1095 ± 240 (control)]. At both survival times, AChE(+) fibers were present within the nerve grafts.

Our results suggest that over time sciatic nerve transplants lose their ability to maintain viable septal cholinergic neurons even though these transplants contain cholinergic axons. Further studies will examine the mechanisms of this response in detail.

Supported by NIA grant #06648.

433.5

IN VITRO STUDIES OF LC-HIPPOCAMPUS DOUBLE BRAIN GRAFTS GROWN IN OCULO: AN IN VIVO ELECTROCHEMICAL STUDY. M.-T. Su*,[1], T.V. Dunwiddie[2,4], M. Mynlieff[2] and G.A. Gerhardt[2,3] (SPON: T.-H. YIN). Dept. of Pharmacology[1], National Defense Medical Center, Taipei, Taiwan, Republic of China and Depts. of Pharmacology[2] and Psychiatry[3], University of Colorado Health Sci. Ctr., Denver, CO 80262 and Medical Research[4], Veterans Administration Medical Center, Denver, CO 80220.

Double grafts of fetal brain tissue have been shown to survive and grow in the anterior eye chamber of the rat. In particular, locus coeruleus (LC) when co-grafted with hippocampus has been previously shown to invade the hippocampal tissue with an organotypic norepinephrine innervation that appears to function similarly to the normal LC-hippocampal projection. In the present study, LC-hippocampus double grafts which had been grown in oculo were removed from the host and placed in a standard brain-slice recording chamber. High-speed electrochemical measurements and pressure-ejection of KCl were employed to investigate the connectivity of the grafts. Local ejections of potassium into the LC portion of the double graft elicited overflow of NE in localized regions of the hippocampal portion of the double grafts. In addition, superfusion of the grafts with both phentolamine and nomifensine caused an augmentation in the potassium-induced responses. These data demonstrate that LC graft neurons are able to release NE in target tissues when stimulated in an appropriate manner. Supported by USPHS AG06434, AG04418, AG00441 and the VA Research Service.

433.6

NEURAL TRANSPLANTATION IN ADULT RATS WITH EARLY RADIATION-INDUCED HYPOPLASIA OF FASCIA DENTATA GRANULE CELLS: SELECTIVE ATTENUATION OF BEHAVIORAL DEFICITS G. Andrew Mickley, J. Leland Ferguson, Thomas J. Nemeth* and Maureen A. Mulvihill.* BHS, AFRRI, Bethesda, MD 20184-5145 USA.

Partially shielded rat brains were X-irradiated during the first 16 days post partum. This procedure depleted 90% of the hippocampal granule cells while sparing other brain areas. When subjects were 147 ± 4 days of age, we conducted behavioral tests sensitive to hippocampal damage. Irradiated subjects later (average age = 182 ± 4 days) received intra-cerebral transplants from either fetal fascia dentata or cerebral cortex. Controls (both irradiated and sham-irradiated) experienced no surgery or sham-surgical procedures. Subjects were retested at 265 ± 5 and 350 ± 6 days of age. Both hippocampal and cerebral cortex grafts significantly reduced performance deficits on a passive avoidance task. Hippocampal transplants (especially those located in the damaged hippocampus) also significantly attenuated perseverative spontaneous rotation. Thus, selected behavioral deficits associated with early fascia dentata damage may be attenuated through the use of neural grafting in mature subjects. An assessment of graft-induced behavioral benefits depends on (1) the time after transplantation that particular behavioral tests are conducted, and (2) the source and final location of the grafted neural tissue.

433.7

THE RELATIONSHIP OF FETAL SEPTAL GRAFT TO SPATIAL LEARNING IN RATS WITH A TRIMETHLTIN-INDUCED HIPPOCAMPAL LESION. N.Kato, M.Akaike*, A.Masui* and T.Kobayashi* Dept. of Psychiat., Shiga Univ. Med. Sci., Otsu 520-21 and Pharma Res. Labs., Hoechst Japan Ltd., Kawagoe 350, Japan.

The spatial learning impairments in rats with a hippocampal lesion induced by single oral administration of trimethyltin hydroxide (TMT,9mg /kg) were examined and compared with those in TMT-treated rats with fetal septal grafts. TMT-treatment induced a permanent increase in open-field activity 3 days after the treatment and increased number of errors as detected by Biel water maze which appeared as early as 4 hr and reached to the steady level 5 days after the administration. Rats were treated with TMT followed a week later by implant of septal tissues taken from 14 day fetuses and digested by trypsin into bilateral 3 regions of the hippocampus. Spatial learning was evaluated by Morris water maze test 9 weeks postoperatively. The swimming time to find a submerged platform over 10 daily trials was recorded for 2 days. TMT-treated rats with sham operation exhibited a longer time spent in the water as compared with control rats and TMT-treated rats receiving septal grafts showed improved performance relative to TMT-rats, though still inferior to controls.

433.8

TRANSPLANTATION OF FETAL CHOLINERGIC NEURONS PROMOTES RECOVERY OF AF64A-INDUCED BEHAVIORAL AND NEUROCHEMICAL DEFICITS. T.J. Walsh, Black, B. and D.F. Emerich. Rutgers University, Department of Psychology, New Brunswick, N.J. 08903.

The studies presented here examined the effects of transplanted fetal cholinergic neurons on the behavioral and neurochemical alterations induced by AF64A. This cholinotoxin produces long-lasting decreases in high affinity choline uptake levels (HAChU) and the choline acetyltransferase (ChAT) activity in the hippocampus (HPC) together with pesistent cognitive impairments.

Sprague-Dawley rats were trained on a standard eight arm radial maze (RAM) task. Following training, rats received bilateral injections of AF64A (3 nmol/side/icv). Ten days later, suspensions of dissociated septal cholinergic neurons (E16) or the glucose-saline vehicle (VEH) were injected into the HPC at two rostrocaudal locations. Rats injected with AF64A (AF/VEH) were markedly and permanently impaired in their performance of the RAM task. In contrast, animals receiving transplants (AF/TR) were initially impaired but reacquired the task within 60 trials.

Rats were then trained to perform a working memory version of the RAM task in which they had to remember which 4 of the 8 maze arms they obtained food from prior to a one hour delay. Following the delay the rats were returned to the maze and allowed to choose freely among all 8 arms. Arms not previously chosen were baited and entry into previously entered arms constituted an error (delayed-non-match-to-sample). Both AF64A-treated groups (AF/VEH and AF/TR) were impaired on this version of the task and showed no evidence of recovery. AF64A produced decreases in hippocampal HAChU levels (31%) and ChAT activity (35%) that was attenuated (14 and 27%) in the AF/TR group.

These data suggest that the transplantation of fetal septal cholinergic neurons following AF64A promotes functional recovery in a task-dependent manner and that this recovery is associated with an attenuation of the AF64A-induced cholinotoxicity.

Supported by BRSG Grant 07058 to TJW.

433.9

FETAL HIPPOCAMPAL TRANSPLANTS AMELIORATE SPATIAL MEMORY DEFICITS IN MONGOLIAN GERBILS WITH ISCHEMIA-INDUCED DAMAGE TO THE HIPPOCAMPUS. S. M. Onifer and W. C. Low. Department of Physiology and Biophysics. Indiana University School of Medicine, Indianapolis, IN 46223.

Cerebral ischemia produces a selective loss of CA_1 pyramidal neurons in the dorsal hippocampus in the Mongolian gerbil resulting in spatial memory deficits. The purpose of this study was to determine the effects of fetal hippocampal cells transplanted into the ischemic hippocampus on the restoration of spatial memory function. Transient cerebral ischemia was produced by bilateral carotid artery occlusion for 10 minutes. Ischemic and non-ischemic gerbils were trained in a Morris water-maze to swim to a hidden platform followed by a spatial probe test.

The number of platform crossovers (mean $\pm$ S.E.M.) in the training quadrant prior to and after transplantation was determined for each group. Ischemic gerbils with sham transplants showed no improvement in spatial probe behavior after transplantation (6.8$\pm$0.8 vs. 6.0$\pm$0.8, $p>0.05$). Similarly, no improvement was observed in sham-operated gerbils (12.7$\pm$0.9 vs. 10.5$\pm$1.1, $p>0.05$). In contrast, ischemic gerbils with hippocampal transplants exhibited a significant improvement in spatial probe behavior after transplantation (4.3$\pm$1.3 vs. 8.8 $\pm$1.2, $p<0.05$).

Bilateral degeneration of the host's CA_1 pyramidal neurons was observed in both the ischemic group with transplants and in the ischemic group with sham-transplants. In animals with grafts, transplanted cells, morphologically similar to CA_1 pyramidal neurons, were found in the CA_1 field. Frequently, they were in aggregates in stratum pyramidale, oriens and radiatum of regio superior and also individually scattered throughout the CA_1 field. Clusters of transplanted cells were also found dorsal to the hippocampus and partially embedded within the corpus callosum. These grafts were in contact with the hippocampus via penetrations through the alveus. Host-derived AChE-positive fibers innervated the grafts through these alvear penetrations and from the cortex. These data suggest that fetal hippocampal cells, morphologically similar to CA_1 pyramidal neurons, will survive when transplanted into the CA_1 field in the ischemic hippocampus of the Mongolian gerbil and are innervated by host-derived AChE-positive fibers. These grafted cells may in turn ameliorate deficits in spatial memory that result from cerebral ischemia. (Support by AHA and AHA, Indiana Affiliate, Inc.)

433.10

RELATIONSHIP OF HOST DEAFFERENTATION AND EMBRYONIC TRANSPLANTATION IN THE REINNERVATION OF ENTORHINAL TERRITORY IN THE ADULT MOUSE DENTATE GYRUS. C.-F. Zhou*-, Y. Li*-, P. M. Field* and G. Raisman, Lee Research Centre, Lab of Neurobiology, NIMR, London NW7 1AA, UK, and -Shanghai Physiology Institute, PR China.

Thy-1.1 to Thy-1.2 mouse congenic transplants were used to show that embryonic entorhinal area projects specifically to the appropriate entorhinal afferent territory of the outer two-thirds of the stratum moleculare of the adult host dentate gyrus. The projection requires that the terminal field is deafferented by removal of the host entorhinal area. Deafferentation is effective if carried out at the time of transplantation or for up to 2 months before transplantation. This indicates that for 2 months after host entorhinal lesions, host sprouting either does not occur or is not sufficient to prevent ingrowth of transplant entorhinal axons. Quantitative electron microscopic studies show the relationship between transplant and sprouting host fibres in their capacity to reinnervate the denervated entorhinal territory.

433.11

SELECTIVE INNERVATION OF CA3 HIPPOCAMPAL TRANSPLANTS BY ADULT HOST DENTATE GRANULE CELL AXONS. P.M. Field*, P.J. Seeley*, M. Frotscher-* & G. Raisman (SPON: Brain Research Association), Lee Research Centre, Lab. of Neurobiology, National Institute for Medical Research, London NW7 1AA, UK and -Dept of Anatomy, University of Frankfurt, FRG.

We have studied formation of characteristic mossy fibre synapses between granule cell axons of adult PVG rat hosts and embryonic transplants from syngeneic rat hippocampus. Late embryonic hippocampus was subdissected into its proto-cytoarchitectonic fields and the quality of this subdissection monitored from the cytology of neurons in semi-thin sections, birthdate analysis using tritiated thymidine, staining with an antibody (py) that is selective for large pyramidal neurons of CA3, and EM analysis of identified Golgi stained CA3 pyramids and mossy fibres.

Transplanted large pyramidal neurons receive mossy fibre synapses only when the transplant makes direct contact with the host mossy fibre system and these connections are selective for CA3 compared with CA1 pyramids.

433.12

CONNECTIONS OF THY 1.2 LABELED CROSS-SPECIES TRANSPLANTS IN THE DENTATE GYRUS USING EM. B.P. Vietje, J. Wells, and R. J. McKeon. (SPON: J. Held) Department of Anatomy and Neurobiology, University of Vermont, Burlington, VT 05405.

Neural cell suspensions from the septal/basal forebrain region of Thy 1.2-positive mouse embryos were transplanted into the hippocampus of Thy 1.1 rat hosts. A Thy 1.2 antibody was found on the external membranes and certain internal membranes of the transplanted neurons. Within the transplant, labeled presynaptic profiles contacted labeled postsynaptic profiles. Many unlabeled presynaptic profiles contacted labeled postsynaptic profiles. Most were asymmetric synapes on dendrites of a variety of diameters. In the host close to the transplant, the labeled fibers growing out of the transplant were often fasciculated into large groups. Each fascicle was surrounded by an unlabeled astrocytic process but there were no unlabeled processes within the fascicles. Further away from the transplant few fascicles were seen but labeled individual fibers were seen coursing through the host neuropil. There was not a consistent orientation between the labeled distal fibers and astrocytes, but a few labeled presynaptic profiles also were seen contacting unlabeled postsynaptic profiles of the host. Supported by PHS 23266.

433.13

GROWTH OF CHOLINERGIC SEPTAL-BASAL FOREBRAIN TISSUE FOLLOWING CROSS-SPECIES TRANSPLANTATION. R.J. McKeon, B.P. Vietje*, and J. Wells. Department of Anatomy and Neurobiology, University of Vermont, Burlington, VT 05405.

Cell suspensions of septal-basal forebrain tissue from embryonic Thy 1.2-positive mice were transplanted to the dentate gyrus of cholinergically denervated Thy 1.1-positive rat hosts. Antibodies to Thy 1.2 and ChAT were used to compare the distribution of the donor tissue and ChAT-positive cells and processes following transplantation. Both Thy 1.2-positive and ChAT-positive cells and processes were similarly distributed within the host dentate gyrus. ChAT-positive staining was most dense within the transplant and ChAT-positive cells and processes were clearly evident within the hilus and intact molecular layer. The ChAT-positive cells and processes were determined to be of donor origin since they were not present in sections of normal or lesion control animals. Thus, ChAT immunocytochemistry corroborates the selective growth of xenogeneic Thy 1.2-positive tissue. Additionally, neither the Thy 1.2 nor ChAT staining matches reports of AChE staining following xenogeneic transplants. These data suggest that the AChE-positive ingrowth within the host following xenogenic transplants is derived from both the donor and the host. Supported by PHS 23266.

433.14

INCREASE OF ACHE IN THE MOLECULAR LAYER OF THE DENTATE GYRUS AFTER GRANULE CELL LESIONS IN THE ABSENCE OF EXTRINSIC ACHE INPUTS. J. Wells, R. J. McKeon and B. P. Vietje. Department of Anatomy and Neurobiology, University of Vermont, Burlington, VT 05405.

Selective lesions of the granule cells of the dentate gyrus were made by fluid injection into the infragranular cleavage plane. The animals were sacrificed at various times after the lesions and sections were stained histochemically for AChE and immunocytochemically for choline acetyltransferase (ChAT). The AChE increased markedly in the molecular layer above the granule cell lesion but was unchanged in the molecular layer away from the lesion. The increase in AChE also was seen after combined granule cell and fornix lesions but no AChE was present in animals with a fornix lesion alone. The portion of the molecular layer which stained densely for AChE did not react with an antibody to ChAT. We suspect that the non-cholinergic but AChE-positive interneurons that normally project to the molecular layer have sprouted in response to the loss of their usual target. The molecular layer also shrinks after the granule cell lesion. The shrinkage may add to the dense appearance of the AChE but the AChE increase occurred independently of the shrinkage. Supported by PHS 23266.

433.15

FETAL MONKEY BASAL FOREBRAIN NEURONS GRAFTED INTO NUCLEUS BASALIS LESIONED CEBUS MONKEYS. J.H. Kordower[1], M.S. Fiandaca, and D.M. Gash. [1]Dept. of Anatomy and Cell Biology, Univ. Illinois Sch. Med., Chicago Ill. 60302. USA.

Grafts containing cholinergic neurons derived from the embryonic rodent basal forebrain have been demonstrated to survive transplantation and ameliorate some of the memory deficits which result from aging processes or lesions of either the septohippocampal pathway or nucleus basalis. To date, no studies have examined the extent to which such grafts survive in the primate CNS. Four young adult (1.5-2.5 kg) male *Cebus apella* monkeys received unilateral ibotentic acid lesions of the nucleus basalis. Six injections of this toxin were made in 1.2 µl volumes at a concentration of 10 µg/µl. Seven to ten days later, monkeys received cortical and amygdaloid implants of basal forebrain neurons obtained from E60 (n=2) or E90 (n=2) *Cebus apella* fetuses. All monkeys were sacrificed 10 weeks later. Nissl staining, nerve growth factor (NGF) receptor immunocytochemistry, and acetylcholinesterase (AChE) and diophorase (NADPH) histochemistry revealed comprehensive unilateral lesions of the nucleus basalis. Ipsilateral to the lesion, cholinergic fiber staining was clearly reduced within the external capsule and cortex and virtually eliminated within the amygdaloid basolateral nucleus. In monkeys receiving implants from the E90 fetus, few surviving grafted neurons were observed. In contrast, numerous grafted magnocellular, NGF receptor-immunoreactive and AChE positive neurons were observed in monkeys receiving E60 grafted tissue. The neurons were organized in clusters in a manner similar to that observed *in situ*. Long processes emanating from these cells abutted, but did not cross, the graft/host interface. In some instances, healthy appearing Nissl stained grafts failed to contain cholinergic neurons. Numerous NADPH-containing neurons with morphological characteristics similar to those seen within the Cebus basal forebrain were observed in these transplants. These data indicate that both cholinergic and noncholinergic fetal monkey basal forebrain neurons survive well following grafting into the primate CNS. (Support: NS25655 and The American Health Assistance Foundation (JHK)).

433.16

FETAL CHOLINERGIC GRAFTS TO THE BASAL FOREBRAIN IN THE AGED RAT. K.S. Chen*, G. Buzsaki, M. Benoualid*, and F.H. Gage. (SPON: R.T. Boyd) Dept. of Neuroscience (M-024), UCSD, La Jolla, CA 92093.

Rats exhibit behavioral, electrophysiological, and anatomical changes as a function of aging. Fetal grafts of basal forebrain cells into the hippocampus and/or cortex have been shown to ameliorate some of these age-related changes. In this study we implanted 3ul cell suspensions grafts of the basal forebrain region of fetal rats (E14-E16) bilaterally into the area of the nucleus basalis of aged (24 mos) Fischer 344 rats. These aged rats had been behaviorally characterized on the Morris water maze and exhibited an impairment on this task. Upon retesting on this task following implantation of the fetal grafts, the rats that had received grafts had significantly improved their performance to the level of aged rats that did not initially exhibit an impairment on the water maze task. The impaired aged rats that did not receive grafts did not show an improvement.

Aged rats also exhibit pathological EEG patterns as reflected by frequent long-duration high voltage neocortical spindles that did not correlate with their performances on the water maze. Graft survival was demonstrated by acetylcholinesterase (AChE) staining. Immunohistochemical staining for choline acetyltransferase (ChAT) and NGF receptor (NGFr) revealed the presence of ChAT-positive and NGFr-positive cells in the grafts. These results suggest that grafts of fetal cells to the nucleus basalis region survive well in the aged rat, and may be able to reverse some age-related functional deficits.

DRUGS OF ABUSE: CNS PATHWAYS

434.1

LACK OF TOLERANCE TO NICOTINE-INDUCED DOPAMINE RELEASE IN THE NUCLEUS ACCUMBENS OF RATS: AN *IN VIVO* MICRODIALYSIS STUDY. J. Day, G. Damsma and H.C. Fibiger. Div. of Neurological Sciences, Dept. of Psychiatry, University of British Columbia, Vancouver, B.C. V6T 2A1.

The extent to which repeated administration produces tolerance to nicotine-induced increases in dopamine transmission in the nucleus accumbens was investigated in rats. *In vivo* microdialysis was used to sample extracellular concentrations of dopamine and metabolites after a nicotine challenge (0.35 mg/kg) in (1) naive rats, (2) acutely pretreated rats (one prior nicotine injection), and (3) chronically pretreated rats (twelve to fifteen prior daily nicotine injections, 0.35 mg/kg/injection). Nicotine increased extracellular concentrations of DA and its metabolites, and these increases were not significantly altered by either acute or chronic prior exposure to the drug. The failure to find evidence of tolerance is compatible with the hypothesis that the mesolimbic dopaminergic system is a substrate for the reinforcing properties of chronically administered nicotine.

434.2

PROFILE OF THE EXTRACELLULAR CONCENTRATION OF COCAINE AND DOPAMINE UNDER SELF-ADMINISTRATION CONDITIONS. **H.O. Pettit, H. Pan* and J.B. Justice Jr.; Emory University; Dept. of Chemistry; Atlanta, Georgia 30322.**

Repetitive administration of cocaine leads to a buildup of both cocaine and dopamine (DA) concentrations in the extracellular fluid of the brain. A series of experiments were undertaken to monitor cocaine and DA concentrations in the nucleus accumbens under a variety of cocaine administration conditions. In vivo microdialysis coupled to high pressure liquid chromatography with ultraviolet detection or electrochemical detection was used to analyze cocaine and DA concentrations, respectively. Cocaine concentrations were measured following the intravenous administration of (1) a single infusion of 0.75 mg/kg cocaine; (2) 0.75 mg/kg/inj of cocaine delivered in five minute intervals; and (3) 1.5 mg/kg/inj of cocaine delivered in eight minute intervals. Concentrations of DA were quantified in animals during the intravenous self-administration of either 0.25, 0.5 or 0.75 mg/inj of cocaine (doses correspond to 0.75, 1.5 and 2.25 mg/kg/inj). Cocaine concentrations were observed to increase and stabilize during repetitive administration in a dose dependent manner. Cocaine concentrations were estimated to be approximately 20 and 30 μM for 0.75 and 1.5 mg/kg/inj doses, respectively. The extracellular concentration of DA was also observed to increase and stabilize at approximately 400% of basal levels during cocaine self-administration. Results indicate that extracellular cocaine and DA concentrations are maintained at an increased, but steady level by a repetitive cocaine administration schedule.

434.3

EFFECTS OF PHENCYCLIDINE (PCP), A NON-COMPETITIVE, AND NPC 12626, A COMPETITIVE N-METHYL-D-ASPARTATE ANTAGONIST ON MESOLIMBIC DOPAMINE SYSTEMS: AN ELECTROPHYSIOLOGICAL AND BEHAVIORAL COMPARISON. S.Levenson* and E.D.French, Dept. Pharmacology, Univ. Arizona, Coll.Med. Tucson, AZ 85724.

We have found that the potency of PCP-like drugs to activate VTA dopamine (DA) neurons is correlated positively to their potency as non-competitive NMDA receptor antagonists. However, aside from some behavioral effects similar to PCP, there is no data to indicate that competitive NMDA blockers will activate mesocorticolimbic DA neurons. These A10 cells are often considered the probable substrate for PCP's psychotomimetic effects.

Extracellular recordings from single VTA A10 neurons were made in chloral hydrate anesthetized rats during i.v. drug injections. From these recordings cumulative dose-response curves were established for MK-801, PCP and NPC 12626. MK-801 and PCP elicited maximum increases in neuronal firing of 40% at 0.5 and 1 mg/kg, respectively, while NPC 12626 altered firing only 4% with doses up to 100 mg/kg. The lack of an NPC effect would not appear to be due to limited access to the CNS since i.p. NPC (50 mg/kg) can produce locomotor hyperactivity and ataxia like that seen with PCP. In order to determine whether the behavioral effects, like those of PCP, were mediated via mesolimbic DA systems animals received bilateral intra-accumbens injections of 6-hydroxydopamine (4 ug/ul) or vehicle 14 days before challenge injections of NPC (50 mg/kg), PCP (5 mg/kg), or d-amphetamine (1.5 mg/kg) and activity measured in photocell cages. Unlike PCP and d-AMP, NPC's locomotor stimulatory effects were not blocked in 6-OHDA lesioned animals.

These results suggest that, in contrast to PCP-like psychotomimetic drugs, competitive NMDA antagonists do not stimulate A10 neuronal activity nor activate locomotor behavior via mesolimbic DA systems.

434.4

ACCUMBENS DOPAMINE LESIONS PRODUCE A TEMPORARY EXAGGERATION IN BEHAVIORAL ACTIVITY FOLLOWING OPIATE MICROINJECTIONS. P. Johnson*, G. Alsterberg*, J.R. Stellar (SPON: A. Skanveski). Psychology Department, Northeastern Univ., Boston, Ma., 02115.

Stinus et al. (Psychopharm. 85:323, 1985) have shown that following 6-OHDA lesions of the nucleus accumbens, microinjection of D-Ala-Met-enkephalinamide (DALA) produces a greater increase in behavioral activity than seen in pre-lesion baseline.

In 5 subjects receiving a DALA injection each week for 5 weeks, we report a similar increase in DALA-induced behavioral activation peaking at 632% (±169) of pre-lesion baseline 2 weeks after lesion, but we also report a reduction in this DALA increase to 164% (±71) by 5 weeks post-lesion. Baseline behavioral activity (no DALA) also increased and decreased over this period post-lesion peaking at 327% (±19) in the 2nd-3rd week after lesion. Current work concerns attempts to identify the mechanisms of this temporary increase and application to rewarding lateral hypothalamic self-stimulation.

Supported by the Whitehall Foundation

434.5

CHRONIC COCAINE ADMINISTRATION ENHANCES EXTRACELLULAR CONCENTRATIONS OF COCAINE AND DOPAMINE FOLLOWING A CHALLENGE INJECTION. **H. Pan*, H.O. Pettit and J.B. Justice Jr.; Emory University; Dept. of Chemistry; Atlanta, Georgia 30322.**

Chronic cocaine administration produces significant increases in behavioral responses to a cocaine challenge injection. Cocaine-induced locomotor activity and stereotypy are enhanced in animals that receive daily cocaine injections. Supersensitive post-synaptic receptors, a down regulation of autoreceptors, and/or conditioning effects have been proposed to explain reverse tolerance effects of chronic cocaine administration. The present study used in vivo microdialysis techniques to monitor extracellular concentrations of both dopamine (DA) and cocaine in two groups of animals (N = 4/grp). One group of animals (acute group) received one subcutaneous (s.c.) injection of saline per day for 10 consecutive days. The other group of animals (chronic group) received one s.c. injection of cocaine per day for 10 consecutive days (days 1-5, 10 mg/kg; days 6-10, 20 mg/kg). On day 11, each animal was anesthetized, placed in a stereotaxic instrument and a microdialysis probe was lowered into the nucleus accumbens. Once baseline measurements were obtained, each animal received an intraperitoneal injection of cocaine (30 mg/kg). Cocaine and DA concentrations were quantified by microbore HPLC with ultraviolet or electrochemical detection, respectively. Both cocaine and DA concentrations were significantly higher in animals that received chronic vs acute cocaine administration (>200%). Results indicate that reverse tolerance effects observed following chronic cocaine administration may be due to significantly higher cocaine concentrations. Furthermore, the increase in DA concentration observed in chronic animals was higher than could be accounted for by the increase in cocaine concentration. Results indicate two physiological changes as a result of chronic cocaine administration: one that increases extracellular cocaine concentrations; and another that enhances the ability of cocaine to increase the extracellular concentration of dopamine.

434.6

IN VIVO BRAIN MICRODIALYSIS STUDIES OF Δ^9-TETRAHYDROCANNABINOL ON PRESYNAPTIC DOPAMINE EFFLUX IN NUCLEUS ACCUMBENS OF THE LEWIS RAT. J. Chen*, W. Paredes*, J. Li*, D. Smith*, and E.L. Gardner. Depts of Neuroscience and Psychiatry, Albert Einstein College of Medicine, New York, NY 10461.

Marijuana is the most widely used illicit drug in North America, yet the neurobiological substrates for its abuse liability remain unclear. Due to the postulated role of CNS dopamine (DA) systems in mediating the rewarding properties of drugs of abuse (Spyraki et al. Psychopharmacology 79:278 1983), it is possible that Δ^9-tetrahydrocannabinol (THC), the psychoactive ingredient of marijuana, may have agonist-like DA effects. In fact, we previously showed that THC acutely facilitates brain-stimulation reward in rat medial forebrain bundle (Gardner et al. Psychopharmacology 96:142, 1988), known to be sensitive to DA modulation, and produces acute augmentation of K^+-evoked presynaptic DA efflux in rat neostriatum (Ng Cheong Ton et al. Brain Res. 451:59, 1988). We have now carried out in vivo microdialysis studies of THC's effect on basal presynaptic DA efflux in the nucleus accumbens (Acc), a crucial anatomic convergence of brain-reward-relevant DA circuitry. Pharmacologically meaningful low doses of THC produced dose-dependent augmentation of basal DA efflux in Acc peaking at 20-40 mins post-THC and lasting approx. 1 hr; this augmentation was significantly attenuated by naloxone. We suggest that THC is not as anomalous a drug of abuse as some have suggested, and shares with other abused drugs a DA agonist-like action on reward-relevant mesotelencephalic DA circuits.

434.7

SIMULTANEOUS BEHAVIORAL AND NEUROCHEMICAL MEASUREMENTS DURING MICRODIALYSIS INFUSION OF COCAINE AND AMPHETAMINE IN THE NUCLEUS ACCUMBENS OF RATS **S.E. Hemby, H. O. Pettit, D.B. Neill, and J.B. Justice Jr. Departments of Psychology and Chemistry, Emory University, Atlanta, GA 30322**

The increase in locomotor activity and reinforcing effects of psychomotor stimulants are believed to be primarily mediated by dopamine (DA) transmission in the nucleus accumbens (N ACC). Rats will self-administer amphetamine (Hoebel et al., *Psychopharmacology*, 81:158-163,1983), but not cocaine (Goeders & Smith, *Science*, 221:773-775,1983) into the N ACC. Therefore, we examined the locomotor effects and the dopaminergic response in the N ACC during direct infusions of amphetamine and cocaine in the N ACC. Amphetamine (0, 27, 54, and 108mM) or cocaine (0, 118, 235, and 470 mM) was constantly infused through a microdialysis probe into the N ACC. Simultaneously, extracellular DA concentrations were quantified by HPLC-EC analysis of the dialysate every five minutes for two hours. Extracellular DA concentrations increased following cocaine and amphetamine infusions, while sustained locomotor activity was present only in the amphetamine infused rats. Subsequently, we examined the ability of cocaine infused through a microdialysis probe to affect the extracellular concentration of DA 2.0mm away from the infusion site and found increases in extracellular DA concentrations, indicating that a substantial area of the N ACC was affected. These results suggest that cocaine infused directly into the N ACC results in increased extracellular DA concentrations, but does not produce the expected behavioral effects.

434.8

EARLY COCAINE ABSTINENCE ALTERS GLUCOSE UTILIZATION IN DOPAMINERGIC REWARD PATHWAYS. D.W. Clow, S.J. Lee*, D. Yoshishige*, L.J. Porrino and R.P. Hammer, Jr. Dept. Anatomy & Reprod. Biol., Univ. Hawaii Sch. Med., Honolulu, HI 96822 and NINDS, Bethesda, MD 20892.

Chronic cocaine exposure may deplete neuronal dopamine (DA) stores by inhibiting reuptake. Systemic administration of DA receptor agonists, such as bromocriptine (BROM), could compensate for a subsequent reduction of DA output. We investigated the effects of chronic cocaine, followed by a brief period of abstinence (with or without BROM treatment), on local cerebral glucose utilization (lCGU) in rat brain.

In the first study, adult male rats were injected daily with 10 mg/kg cocaine HCl or vehicle i.p. for 14 days, and in the second study, this was followed by a three day period of cocaine abstinence in which the animals were injected daily with 10 mg/kg BROM or vehicle. Animals were then prepared for quantitative analysis of lCGU using [^{14}C]2-deoxyglucose. LCGU experiments began 10 min following cocaine administration (first study) or 2 hr following BROM administration (second study). Chronic cocaine resulted in a general increase in lCGU with significant increases in rostral nucleus accumbens (NAc), medial caudate, globus pallidus and substantia nigra (SN). In contrast, chronic cocaine followed by 3 days of abstinence resulted in a general decrease in lCGU in most regions studied, including NAc, SN and ventral tegmental area. Subsequent BROM administration returned lCGU to control levels in some of these regions. Moreover, correlation analysis of lCGU data revealed that metabolic activation of mesolimbic circuits occurs following chronic cocaine treatment, but not saline treatment. These results suggest that dramatic changes occur in dopaminergic reward regions during the period of early cocaine abstinence, and that these alterations may be influenced by dopaminergic pharmacotherapy during this period. (Supported by USPHS Awards DA04081 and NS01161.)

434.9

EFFECTS OF 6-HYDROXYDOPAMINE LESIONS OF THE MESOCORTICAL/MESOLIMBIC PATHWAYS ON NOVELTY-SEEKING BEHAVIOR OF RATS. R.C. Pierce*, A.J. Nonneman, B.A. Mattingly and M.T. Bardo. Dept. of Psychology, University of Kentucky, Lexington, KY 40506.

The effect of a 6-hydroxydopamine (6-OHDA) lesion (8.0 µg in 2 µl per side) of the mesocortical/mesolimbic pathways (A +2mm, L ± 1.5mm, V -7mm relative to bregma) on the novelty-seeking behavior of rats was studied. Animals were habituated to a previously novel chamber thirty minutes daily over four consecutive days. On the fifth day, animals were allowed free access to both the habituated (familiar) and a distinct novel environment. Control animals displayed a novelty-preference as they spent significantly more time in the novel chamber relative to the familiar chamber. Novelty-seeking was blocked in lesioned animals as they showed no preference for either the novel or familiar chamber. Subsequent assays revealed that the 6-OHDA lesion reduced dopamine levels in the nucleus accumbens (67% depletion), striatum (75% depletion) and olfactory tubercles (68% depletion) as compared to controls. These results suggest that novelty-seeking behavior may be mediated by a brain dopamine pathway.

434.10

NEUROLEPTIC-LIKE EFFECTS OF INTRA-ACCUMBENS CHOLECYSTOKININ ON I.V. COCAINE SELF-ADMINISTRATION. F.J.Vaccarino,[1] F.Weiss and G.F. Koob.[2] [1]Depts. of Psychology and Psychiatry, Univ. of Toronto, Canada, M5S1A1; [2]Div. of Preclinical Neuroscience and Endocrinology, Research Institute of Scripps Clinic.

Increased dopamine (DA) transmission in the nucleus accumbens (Acb) is an essential mechanism underlying the rewarding properties of i.v. cocaine reward. Cholecystokinin (CCK) is present in Acb DA terminals as well as non-DA neurons associated with the mesolimbic system. The present experiment examined the possible modulatory effects of intra-Acb CCK on i.v. cocaine self-administration.

Male Wistar rats with bilateral cannulae aimed at the Acb were trained to self-administer cocaine intavenously during daily 3 h sessions. Following stable responding each rat was tested following intra-Acb microinjections of CCK (0, 0.5, 1 and 2 ng). CCK doses were administered in counterbalanced order. A minimum of 3 no pretreatment days separated drug tests.

The results demonstrated that intra-Acb CCK produced a dose-dependent attenuation of cocaine reward as reflected by an increase in responding for i.v. cocaine. The 1 ng CCK dose, which produced approximately a 75% increase in responding, was the most effective dose. These results demonstrate that CCK has neuroleptic-like effects on i.v. cocaine self-administration.

434.11

COCAINE AND BRAIN REWARD DOPAMINERGIC NEURONAL CIRCUITRY: NEUROBEHAVIORAL CONCOMITANTS. F.T. Phelan* and P.A. Broderick (SPON: L. Mitchell). Dept. Pharmacol., CUNY Med. Sch.: Depts. Biol. & Psych., CUNY Grad., NY 10031.

Brain reward is intimately associated with dopamine in mesolimbic neuronal circuitry. We studied the effect of cocaine on presynaptic mechanisms in nucleus accumbens, the terminal neurons for the ventral tegmental (A10) cell bodies. Dopamine in extracellular fluid was studied by in vivo voltammetry. Locomotor activity was studied by Activity Pattern Analysis (San Diego Instr., Calif.). Electrochemical (ELC) measurements of dopamine, before and after subcutaneous administration of cocaine (20mg/kg), were coupled with behavioral locomotor activity studies in freely moving, male, inbred Sprague-Dawley rats (350-450g). We then further studied the effect of cocaine on extracellular dopamine in striatum and again simultaneously recorded locomotor activity. The results show that the dopamine signal after cocaine, increased in nucleus accumbens of freely moving rats. The increase in dopamine was slow and progressive. By comparison, dopamine in striatum showed less change after cocaine administration. Interestingly, the dopamine alterations occurred before locomotor activity changes began. These data confirm cocaine's action in dopaminergic mesolimbic circuitry.(Spons.:DHHS, PHS Grant #2-S07-RR07132, PSC/CUNY Award #667234 and BRSG#442248 to P.A. Broderick.)

434.12

OPIATES INFLUENCE NUCLEUS ACCUMBENS NEURONAL ACTIVITY BY DOPAMINE AND NON-DOPAMINE MECHANISMS S.J. Henriksen and R.L. Hakan. Department of Neuropharmacology, The Research Institute of Scripps Clinic, La Jolla, CA 92037.

The nucleus accumbens septi (NAS) of the telencephalon is thought to mediate, in part, rewarding components of drug self-administration. Dopamine-containing cell bodies originating in the ventral tegmental area of Tsai (VTA) are a major afferent to the NAS. While VTA-derived dopamine has been implicated in psychostimulant reward, the role of this amine for the rewarding effects of opiates is unclear. We have investigated the effects of systemic and local infusions of morphine on spontaneous and evoked single unit activity of NAS neurons in anesthetized rats. Systemic or iontophoretically applied morphine generally inhibit randomly encountered spontaneously active NAS cells. However, phoretically or systemically applied morphine are uniformly ineffective in altering fimbria-driven NAS activity recorded from normally "silent" NAS units. On the other hand, micro-infusion of morphine (2.5 µg in 1 µl) into the VTA (VTA-M) inhibits this fimbria-driven unit discharge, and subsequent administration of morphine (s.c.) can reverse this VTA-M inhibition suggesting an opposing action of opiates at a separate, non-VTA site. In fact, micro-infusion of morphine into the ventral subiculum, but not into the amygdala, reverses the inhibitory effect of VTA-M on fimbria-evoked NAS activity. These data suggest that the effects of systemic morphine on NAS unit activity result from the integration of opiate-sensitive NAS afferents having potentially opposing actions on NAS activity. (Supported by DA03665, KO2/DA00131 and AA07456 to S.J.H.)

434.13

A METHOD FOR RECORDING SINGLE UNIT ACTIVITY DURING I.V. SELF-ADMINISTRATION OF DRUGS IN THE FREELY MOVING RAT. L.L. Peoples*, M. Wolske, S.I. Dworkin, J.A. Smith, S.A. Deadwyler, M.O. West. Dept. Psychol, Rutgers U., New Brunswick, NJ; Dept. Physiol/Pharm, Bowman Gray Sch. Med., Winston-Salem, NC

The mesocorticolimbic dopamine system has been implicated as an important neural substrate of psychomotor stimulant self-administration. The present experiments were undertaken to electrophysiologically record single unit activity in the nucleus accumbens of rats self-administering cocaine. Male Long-Evans rats (300g) were permanently implanted with microwires (25 micron diam.) in the nucleus accumbens, with a stimulating electrode in the ipsilateral fimbria, and with a catheter in the jugular vein. That recorded units were accumbens neurons was verified by stimulating the fimbria to evoke unit discharges at monosynaptic latencies (i.e., 4-11 msec) and by histological analysis. Computer-sychronized videotape analysis allowed the measurement of neural activity time-locked to particular behavioral events. During experimental sessions, each lever-press caused an infusion of 0.2 ml 1.65 mg/ml cocaine (i.e., 0.33 mg/infusion). Unit responses to cocaine were analyzed in terms of 1) neural correlations with motor activity (e.g., locomotion, lever-pressing) and 2) initial vs cumulative exposure to the drug. Preliminary results obtained during the shaping of self-administration behavior indicate that accumbens neurons exhibited overall firing rates with low means (0.08 - 0.97 Hz) and high standard deviations (0.65 - 0.87 Hz). Cocaine administration induced small and inconsistent changes in neural activity, typically accompanied by increases in the standard deviation of mean firing rate (perhaps due to induction of sterotypy). The present experiments demonstrate the feasibility of recording unit activity in self-administering rats but indicate the necessity of using appropriate motor and sensory controls. Supported by DA 04551 and RR 07058-21.

434.14

NUCLEUS ACCUMBENS AND AMYGDALA ARE SUBSTRATES FOR THE AVERSIVE STIMULUS EFFECTS OF OPIATE WITHDRAWAL. L. Stinus*, M. Le Moal, and G.F. Koob. I.N.S.E.R.M.U.259, Bordeaux, France, and Research Institute of Scripps Clinic, La Jolla, CA 92037.

Specific brain sites for the classic opiate abstinence syndrome of wet dog shakes, ptosis and teeth chattering appear to be widely represented in the brain. Using a more general motivational test in dependent rats, the brain site most sensitive to the response disruptive effects of intracerebral (IC) administration of the opiate antagonist, methylnaloxonium (MN), was the region of the nucleus accumbens, a site also implicated in the acute reinforcing properties of opiates. The present study examined whether IC administration of MN produced aversive stimulus effects as measured by the formation of place aversions. Rats implanted intracerebroventricularly (ICV) or with bilateral cannulas aimed at the medial dorsal thalamus, periaqueductal gray, ventral tegmental area, amygdala or nucleus accumbens were made dependent on morphine by subcutaneous implantation of two 75 mg morphine pellets. The animals were then subjected to place aversion training by pairing of a distinct environment with a single ICV injection of MN . Results showed that at high doses of MN (1000-2000 nanograms) all sites produced a place aversion. However lower doses (250-500 nanograms) produced significant brain site selectivity with the region of the nucleus accumbens most sensitive. Observational measures taken during the post-injection period with the high dose of MN showed various overt signs of opiate withdrawal at most sites except the amygdala where no classic abstinence signs were observed. Results suggest that the region of the nucleus accumbens, and perhaps the amygdala, are critically involved in the aversive stimulus effects of opiate withdrawal. (This work supported by NIDA grant DA04043.)

434.15

INTRA-ACCUMBENS INJECTION OF THE GLUTAMATE ANTAGONIST GDEE INHIBITS THE LOCOMOTOR-ACTIVATING EFFECTS OF COCAINE AND AMPHETAMINE, BUT NOT CAFFEINE. N.R. Swerdlow[1], L. Pulvirenti[2,3] and G.F. Koob[2]. 1. Dept Psychiatry, UCSD, La Jolla, CA 92093. 2. Scripps Clinic & Res. Fdn., La Jolla, CA 92037. 3. Dept. Neurology, Univ. Pavia, Italy

Studies of the neural substrates of the locomotor-activating properties of psychostimulants have focused on brain dopamine (DA) systems, particularly the mesolimbic dopamine system and its terminal fields in the nucleus accumbens (NAC). Advances in our understanding of the efferent and afferent connections of the NAC suggest that the behavioral effects of psychostimulants might be modulated within the NAC by glutamate afferents originating within allocortex. We examined the effects of blockade of NAC glutamate activity on the locomotor-activating effects of cocaine (COC) and amphetamine (AMP) - drugs that stimulate locomotion by enhancing NAC DA activity - and caffeine (CAF), which stimulates locomotion independent of NAC DA.

Animals were equipped with bilateral cannulae aimed above the NAC. One week later, they were tested for locomotor activity in photocell cages following intra-NAC infusion of the glutamate antagonist GDEE (0-20 µg/side), and peripheral injection of COC (20 mg/kg ip), AMP (0.75 mg/kg sc) or caffeine (20 mg/kg sc). GDEE significantly decreased COC- and AMP-stimulated locomotion, but not CAF-stimulated locomotion.

These results suggest that the locomotor-stimulated effects of drugs that enhance NAC DA activity are modulated by NAC glutamate transmission. Glutamate projections to the NAC from allocortex may modulate the behavioral properties of NAC DA activity.

434.16

MICROINJECTION OF NMDA BUT NOT QUISQUALATE RECEPTOR ANTAGONISTS INTO THE NUCLEUS ACCUMBENS MODULATES INTRAVENOUS COCAINE SELF-ADMINISTRATION IN RATS. L. Pulvirenti[1,2] R. Sung*[1] and G.F. Koob[1] 1. Research Inst of Scripps Clinic, La Jolla, CA 92037. 2. Dept Neurology, Univ. Pavia, Italy.

The dopamine system in limbic structures is a critical neurosubstrate for drug reinforcement. In particular, the nucleus accumbens of the ventral striatum (NAC) seems to play a major role modulating i.v. self-administration of psychostimulant drugs. Recent evidence has shown that allocortical glutamate afferences to the NAC may be involved in regulating motivated behavior.This study was therefore designed to determine whether glutamate neurotransmission in the NAC would affect i.v. cocaine self-administration in rats.

We studied the effect of intra-NAC administration of 2-amino,5-phosphonovaleric acid (APV), and glutamic acid diethyl ester (GDEE), selective antagonists at NMDA and quisqualate receptor subtype, respectively. APV at the doses of 1.5 and 3.0 µg/side induced a decrease in the rewarding value of cocaine, measured as an increase in the number of responses for the drug in 3-hr daily sessions. GDEE at the doses of 10 and 20 µg/side was ineffective.

These results show that the endogenous glutamate activity in the NAC may be of importance for the maintainence of iv cocaine self-administration in rats. Since glutamate afferents to the NAC are thought to originate mainly from the hippocampus and the amygdala, this is the first evidence suggesting allocortical modulation of the NAC function in drug reinforcement. (Supported by NIDA grant DA04398)

434.17

NORCOCAINE INHIBITS THE SPONTANEOUS ACTIVITY OF DORSAL RAPHE (DR) SEROTONIN NEURONS IN THE RAT. Joseph M. Paris, Kathryn A. Cunningham and Joan M. Lakoski, Dept. of Pharmacology and Toxicology, University of Texas Medical Branch, Galveston, TX 77550.

Cocaine has been demonstrated to inhibit the activity of catecholaminergic and serotonergic (5-HT) neurons in the CNS. Norcocaine is the primary oxidative metabolite of cocaine which also inhibits monoamine uptake and stimulates locomotor activity. The present series of experiments were undertaken in order to characterize the possible electrophysiological effects of norcocaine on spontaneously firing DR 5-HT neurons.

DR 5-HT neurons were identified utilizing standard in vivo extracellular single-unit recording techniques in chloral hydrate anesthetized male Sprague-Dawley rats. The cumulative dose of (-) norcocaine to inhibit DR 5-HT cell firing by 50% was 0.5 +/- 0.05 mg/kg, i.v. (n = 11); the mean dose to completely suppress activity was 1.5 +/- 0.22 mg/kg. Single bolus injections (0.5 mg/kg) depressed cell activity 54-92%. In addition, the dopamine and 5-HT_{1A} autoreceptor antagonist, spiperone (1.0 mg/kg, i.v.), was able to reverse norcocaine's inhibitory effects. These data suggest that the previously reported depressant effects of cocaine in the DR may be mediated, in part, via the active metabolite, norcocaine. Microiontophoretic studies are in progress to assess the mechanism of norcocaine's effects. Supported by NIDA DA04296.

434.18

FAILURE OF NALOXONE TO BLOCK THE EFFECT OF COCAINE ON INTRACRANIAL SELF-STIMULATION. P.Z. Manderscheid, R.A. Frank & H.P. Williams*. Dept. of Psychology, Univ. of Cincinnati, Cincinnati, OH 45221.

Midbrain dopaminergic pathways and opioid receptor systems have been implicated in the rewarding effects of intracranial self-stimulation. Evidence points to the ventral tegmental area as the site of the interaction of these two systems:e.g., both cocaine and morphine dose-dependently lower self-stimulation thresholds in animals with VTA electrodes. Recently, Bain and Kornetsky (1987) demonstrated that naloxone (an opiate receptor blocker) effectively blocks the threshold lowering effect of cocaine. The present study investigated the effect of naloxone on cocaine-induced changes on self-stimulation thresholds in an effort to replicate the aforementioned results.

Male Sprague-Dawley rats were implanted with electrodes in the ventral tegmental area. Animals were injected with either saline, cocaine HCl (25 mg/kg IP), or naloxone (4 mg/kg IP) plus cocaine (25 mg/kg IP). Thresholds were determined 15, 75, and 195 min post-injection. At all test periods naloxone failed to block or attenuate the threshold lowering effect of cocaine.

It is evident that the relationship between dopaminergic and opioid systems of reward needs further clarification. One explanation of the difference in results may be attributable to the methods used in determining self-stimulation thresholds.

434.19

LACK OF NEUROCHEMICAL EVIDENCE FOR NEUROTOXIC EFFECTS OF REPEATED COCAINE ADMINISTRATION IN RATS ON BRAIN MONOAMINE NEURONS. S.Y. Yeh* and E.B. De Souza. (SPON: R. Liu) Neurobiology Laboratory, NIDA Addiction Research Center, Baltimore, MD 21224.

There is an ongoing controversy concerning potential neurotoxic effects of cocaine administration in rats on brain monoamine neurons. Repeated cocaine administration in rats (10 mg/kg, i.p., 7 or 10 days) has been reported to produce significant long-lasting decreases in dopamine (DA) synthesis, tyrosine hydroxylase content, and increased ^{3}H-spiroperidol binding in several discrete areas of rat brain (Trulson et al., Brain Res. Bul. 19: 35, 1987) and depletion in levels of DA and its metabolites, DOPAC and HVA in frontal cortex and hypothalamus (Wyatt et al., JAMA, 259: 2996, 1988). On the other hand, others have been unable to demonstrate any long-term reduction in brain monoamines following chronic cocaine administration in rats. (Yeh, Fed. Proc. 404, 1987; Kleven et al., Brain Res. Bul. 21: 233, 1988). These conflicting observations led us to reinvestigate the potential toxic effects of chronic cocaine administration in rats on brain monoamines. Rats were injected with cocaine (20 mg/kg, s.c., b.i.d., 7 days) and sacrificed at 1, 8, 15, and 48 days after the last injection. The contents of DA, norepinephrine, serotonin and their metabolites, assayed by HPLC-EC, in frontal cortex, hippocampus, striatum, hypothalamus, midbrain, pons-medulla and spinal cord were not significantly different from levels in saline-injected control rats. These data suggest that repeated cocaine administration in rats does not produce any long-term depletion in brain catecholamine and serotonin content characteristic of neurotoxic actions of the drug.

434.20

COCAINE: ELECTROPHYSIOLOGICAL CHARACTERIZATION OF EFFECTS IN AMYGDALA NUCLEI. **K.A. Cunningham, P.M. Callahan and H.A. Lekan. Dept Pharmacology/Toxicology, Univ Texas Medical Branch, Galveston, Texas 77550.**

The limbic system may be an important substrate of action for cocaine since this stimulant alters psychological processes mediated by this circuitry, including mood and emotion. Cocaine inhibits the uptake of dopamine (DA), norepinephrine (NE) and serotonin (5-HT), systems which innervate limbic regions such as the amygdala which is sensitive to cocaine-induced "kindling". Using extracellular recording techniques, we have begun to characterize the effects of cocaine on the activity of amygdala neurons of rats anesthetized with chloral hydrate (400 mg/kg,ip). Of 20 neurons recorded to date, 14 were located in basolateral and 6 in central amygdala. The spontaneous activity of these neurons was slow and irregular with an average rate of 1.2 Hz (range: 0.1-8.5 Hz); triphasic (+/-/+) action potentials were most typical. Inhibitory responses to cocaine (0.25-2.0 mg/kg, IV) were observed in 50% of the neurons tested with an average percent inhibition from baseline of 54 ± 8.9 %. Cocaine (IV) excited 30% (6/20) of the neurons (71 ± 34 % excitation). Three neurons were excited then completely depressed (15%) while 1 neuron was unaffected by cocaine. Studies of the mechanisms underlying the effects of cocaine within the amygdala are in progress. **Supported by the John Sealy Memorial Fund.**

435.1

CHARACTERIZATION AND DISTRIBUTION OF ESTROGEN RECEPTOR (ER) IN THE DIENCEPHALON OF THE GRAY SHORT-TAILED OPOSSUM. R.J. Handa, E.W. Rodriguez, C.A. Fox, C.D. Jacobson. Dept. of Anatomy, Loyola Univ., Maywood, IL 60153 and Dept. of Veterinary Anatomy, Iowa St. Univ., Ames, IA 50011.

The Brazilian gray short-tailed opossum is a pouchless marsupial whose young are born sexually undifferentiated. This animal is well suited for developmental studies. Previously, Etgen and Fadem (Gen. Comp. Endo. 66:441;1987) detected ER in the hypothalamus-preoptic area (HPOA). In this study we characterized the ER and determined its distribution in brains of male and female opossums. ER were measured by the in vitro binding of ^{3}H-estradiol to cytosol of brain subregions punched from 300 μ frozen sections. Radioinert R2858 was used to define non-specific binding. Saturation analysis showed a single, high affinity binding site in HPOA with a Kd of 0.1×10^{-9} M. Binding was displaced by E_2, DES and R2858, but not by non-estrogenic steroids. High levels of ER were found in the medial preoptic /periventricular preoptic area (57.7±3.5 fmol/mg protein). Intermediate levels were seen in the ventromedial hypothalamus, medial amygdala and arcuate n./median eminence (29.1±1.3; 26.5±3.4; 19.2±1.5 fmol/mg prot., respectively). No sex differences were observed. The presence of a neural ER and its distribution in the adult opossum brain supports the use of this animal model in studies examining steroid dependent organization of the hypothalamus. Supported by Potts Foundation Award (RJH) and NIH HD 16148 (CDJ).

435.2

AROMATASE ACTIVITY IN THE BRAIN OF THE GRAY SHORT-TAILED OPOSSUM (MONODELPHIS DOMESTICA). Barbara H. Fadem and Neil J. MacLusky Department of Psychiatry, UMDNJ-New Jersey Medical School, Newark 07103 and Division of Reproductive Science, Toronto General Hospital, Toronto, Ontario, Canada.

While brain aromatase activity has been demonstrated in eutherian mammals, studies have failed to demonstrate such activity in the brains of adult marsupials. For the present report, hypothalamus and preoptic area obtained from adult male gray opossums were assayed for aromatase activity. Tissues were homogenized in 10 volumes of PKDE buffer (10mM potassium phosphate, 100mM KCl, 10mM dithiothreitol and 1mM disodium EDTA, pH 7.4). Aromatase activity was assayed in triplicate aliquots of the homogenates by the tritiated water release method, essentially as described by Roselli et al. (Endocrinology 114:192, 1984). Control incubates contained either no tissue, or the same quantities of tissue homogenate in the presence of the specific aromatase inhibitor,4-hydroxyandrostenedione (4-OHA).

Aromatase activity was measurable in both the hypothalamic and preoptic area tissue samples (hypothalamus 202.1 ± 14.22 pmol[3H2]O/g protein/h; preoptic area 77.5 ± 7.9 pmol/g protein/h; Means ± S.E.M., n=5). Tritium release from the [3H]A substrate was inhibited >70% in both tissues in the presence of 40μM 4-OHA. Kinetic studies revealed the reaction to be saturable, but with a Km somewhat higher (~0.1 μM) than that observed for rat brain aromatase (0.02-0.05 μM) using the same substrate. Supported by NSF grant BNS 8616514 to B.H.F.

435.3

PROTEIN SYNTHESIS IN THE VENTROMEDIAL HYPOTHALAMUS IS MODIFIED BY SMALL PULSES OF ESTRADIOL. C.D. Condon, D.M. Lynn*, and E.J. Roy Neural and Behavioral Biology Program, Univ. of Illinois, Champaign, IL 61820.

The role of estrogen and the ventromedial hypothalamus (VMH) in reproductive behavior of the rat has been well documented. A hypothesis of estrogen action in the VMH is that estrogen increases synthesis of specific proteins. The purpose of this investigation is to establish if estrogen's mechanism of action in the VMH is by modulating protein synthesis and if so, what proteins are affected.

Twelve, 60 day old Long Evans female rats were ovariectomized and bilaterally implanted with cannulas dorsal to the VMH (inner cannulas 28 g). After 4 days of recovery, 6 rats were injected with 2 pulses of 4 ug/kg 17ß-estradiol, 12 hrs apart (6 controls received alcohol vehicle). At the time of the second pulse, each rat was infused in the VMH for 4 hrs while unanesthetized and unrestrained with 190 uCi of 35-S methionine and cysteine (1:1). After the end of the infusions, the rats were decapitated and the VMH regions dissected by punch. Protein samples from brain tissues were solubilized and run on 2-dimensional SDS-PAGE electrophoresis. The gels were enhanced by fluorography, dried and then exposed to X-ray film. The resulting protein spots were analyzed by an Olympus Cue-2 densitometry system and the optical densities quantified. The results confirm that estrogen modulates VMH activity by altering protein synthesis. Interestingly, many of the low molecular weight proteins affected by estrogen are significantly reduced by the hormone.

435.4

THE EFFECT OF GONADAL STEROIDS ON THE BEHAVIORAL AND BIOCHEMICAL EFFECTS OF HIPPOCAMPAL SYMPATHETIC INGROWTH. A. Peagler*, M. Goyal*, D. Parsons* and L. Harrell; J. Halsey (SPON), Dept. Neurology, VA and University of Alabama Med. Ctr., Birmingham, Ala. 35294.

Following cholinergic denervation of the hippocampus via medial septal lesions (MSL), sympathetic fibers, from the superior cervical ganglia, grow into the hippocampus. Previous studies have demonstrated a detrimental effect of these fibers on recovery of a spatial-learning task in male but not female animals. In this study we assessed the role of male sex hormones on the behavioral and biochemical effects of sympathetic ingrowth (SI). For the behavioral studies all animals were gonadectomized (GDx) and taught a standard radial 8-arm maze task. Following attainment of criterion animals underwent one of three surgical procedures: sham, MSL, MSL + ganglionectomy. MSL were found to severely impair reacquisition of the task. However, GDx was found to block the detrimental effect of SI. For the biochemical studies hippocampal norepinephrine (NE) and choline acetyltransferase (ChAT) were measured eight weeks after surgery in sham GDx and GDx animals. MSL were found to significantly reduce the ChAT activity, regardless of circulating sex hormones. GDx was found to significantly reduce the level of NE associated with SI, while having no effect on central NE in CON or MSGx animals. These studies suggest that circulating male sex hormones can influence both the behavioral and biochemical processes associated with HSI.

435.5

SEXUAL BEHAVIOR ELICITED BY STIMULATION OF THE MONKEY MIDBRAIN. S. Aou, E. Okada*, A. Takaki*, and Y. Oomura. Natl. Inst. Physiol. Sci., Okazaki 444; Dept. of Physiol. Kyushu University 60, Fukuoka 812, Japan.

The effects of midbrain electrical (0.2 ms, 10-100 Hz, 125 pulses, 50-500 uA) and chemical (0.01-0.1M Na-L-glutamate, 2ul) stimulation on sexual behavior of male and female monkeys were examined. Six male and three ovariectomized, estrogen-treated female rhesus monkeys were used in accordance with the NIH Guide (1985). In three of the six male monkeys, touching, sometimes followed by mounting, was elicited during electrical stimulation of a wide area of the tegmentum; including the red nucleus, substantia nigra and reticular nucleus. Stimulation of the central gray was not effective. The responses were elicited with a mean latency of 1 s and ceased quickly after termination of stimulation. Chemical stimulation of the loci where electrical stimulation was effective, however, failed to elicit sexual behavior. In the female monkeys, two types of presenting responses were evoked with longer latency than was the case for male monkeys after electrical stimulation of the central gray and adjacent tegmentum. One response was elicited with a mean latency of 7 s. The other, with longer latency (>15 s), was a long-lasting repetitive presenting. The present study suggests that different areas of the midbrain are involved in the control of sexual behavior of male and female monkeys.

435.6

CENTRAL GRAY CONNECTIONS WITH THE VENTROMEDIAL HYPOTHALAMUS ARE ESSENTIAL FOR LORDOSIS IN FEMALE RATS. Ann C. Hennessey, Lael Camak* and David A. Edwards, Department of Psychology, Emory University, Atlanta, Georgia 30322.

The central gray is believed to play an important role in the control of sexual receptivity in female rats, but published reports relating central gray destruction to lordosis in female rats describe only modest deficits in sex tests with males. We show that appropriately placed central gray lesions and knife-cuts virtually eliminate lordosis, and we also provide compelling evidence that central gray connections with the ventromedial hypothalamus are essential for female sexual behavior. Details follow.

Electrolytic lesions of the midbrain-pontine central gray (CG) and its lateral surround eliminate lordosis in ovariectomized females injected with estrogen and progesterone. In a second study, sagittal knife-cuts which bracket the central gray at the level of the rostral pons also eliminate lordosis. The central gray is reciprocally connected with the ventromedial hypothalamus (VMH) by axons entering and leaving the central gray in the lateral and medial planes. Females with a sagittal transection lateral to the CG on one side of the brain combined with a contralateral sagittal transection at the level of the VMH almost never show lordosis. The asymmetric transections spare periventricular hypothalamic connections with the CG, but bilaterally destroy the lateral connecting pathway between the VMH and the central gray, and it seems clear that the "lateral pathway" connections between the ventromedial hypothalamus and central gray play an essential role in the control of lordosis. (Supported by NSF grant BNS-8718797)

435.7

ESTROGEN AND PROGESTERONE EFFECTS ON REPRODUCTIVE BEHAVIOR WHEN THE FEMALE RAT CAN AVOID SOCIO-SEXUAL CONTACT.
S. Schwartz-Giblin, J.J. Blackett and D.W. Pfaff.
Rockefeller University, New York, N.Y. 10021.

To establish an assay relevant to reproductive behavior which also tests for the female's disposition to receive somatosensory stimulation, we used a two-chamber apparatus which allows access to the second chamber only by the female rat. The male was tethered in the first chamber; the door leading from the first chamber was partially closed, thus requiring the female to deliberately leave the mating site. Females were ovariectomized and all received s.c. silastics containing 5mm of 10% estradiol (E) in cholesterol. Three hormone-treatment groups were compared: 4 days of E, 14 days of E, and 4 days of E plus 1 mg progesterone (P) s.c. in oil, 4-5 hr before testing.

The E + P -treated females were significantly different from the other two groups. They showed more lordoses per 20 min test ($p = .005$), fewer rejections per 20 min test ($p = .006$) and spent a smaller percent of the time out of the first chamber ($p = .04$). Thus, behavior paced by the female (cf. Emery, Behavioral Neuroscience, 1986) yielded a strong progesterone effect. These baseline data will be used to evaluate drugs relevant to somatosensation.

435.8

EFFECT OF REMOVAL OF THE VOMERONASAL ORGAN ON SEXUAL BEHAVIOR IN FEMALE RATS.
G.Rajendren*, C.A. Dudley, and R.L. Moss (SPON: E. Hollingsworth). Dept. Physiology, University of Texas Southwestern Medical Center, Dallas, Texas 75235

The role of the vomeronasal organ (VNO) on the display of mating behavior was studied in ovariectomized female rats under two experimental conditions. In the first experiment, VNO-removed (n=15) or sham-operated (n=11) females were primed with 2ug estradiol benzoate (EB) followed 42 hours later by 2.5 mg of progesterone (P). Four to six hours after P injection, the females were tested for reproductive behavior. The preference of the females for a sexually active male over a castrated male, proestrous female or an ovariectomized female was not affected by VNO removal. However, the VNO-removed females exhibited less proceptive behaviors and more resistive behaviors than the sham-operated controls ($p<0.005$). Even though the lordosis to mount (L/M) ratio was relatively high in the VNO-removed females (mean 81.2%), it was significantly less as compared with that in the sham-operated controls (mean 96.5%) ($p=0.018$).

In the second experiment, VNO-removed (n=12) or sham-operated (n=13) females were injected with 2ug EB. Beginning at 48 hours after EB priming, the females were tested for sexual receptivity once in every 30 min for a period of 5 hours. The enhancement of sexual receptivity following repeated mating was significantly reduced in the VNO-removed females as compared with that in the controls ($P<0.001$).

The results suggest that the VNO plays a modulatory role in sexual behavior in female rats. Supported by NIH-MH41784.

435.9

FACILITATION OF LORDOSIS IN INTACT, CYCLING FEMALE RATS FOLLOWING INTRAVENTRICULAR INFUSION OF ESERINE DURING PROESTRUS BUT NOT DIESTRUS.
C. S. Menard, G. P. Dohanich, and N. M. Estella. Dept. of Psychology and Neuroscience, Tulane Univ., New Orleans, LA 70118.

Both systemic and intraventricular administrations of the cholinergic muscarinic antagonist, scopolamine, have been shown previously to inhibit naturally occurring sexual behavior in intact, cycling female rats (Menard & Dohanich, Physio Behav 45:in press, 1989). The present study attempted to examine cholinergic facilitation of sexual behavior in intact, cycling female rats. In the first experiment, intraventricular infusion of the acetylcholinesterase inhibitor, eserine (10ug bilaterally), did not facilitate lordotic responding 15 min after administration when infused during Diestrus I, Mid-diestrus, or Diestrus II. In a second experiment, however, intraventricular infusion of eserine did facilitate lordotic responding 15 min ($p<0.0001$) and 1 hr ($p<0.0006$) after administration when infused during Early Proestrus and Proestrus. Cycling was determined by daily monitoring of sexual behavior and vaginal cytology. As previously reported with the administration of other cholinergic agents, infusion of eserine did not significantly interrupt cyclicity patterns. Because estrogen levels are highest during Proestrus and cholinergic facilitation appears to be limited to this time, it is suggested that estrogen priming of central cholinergic systems is necessary for the cholinergic regulation of sexual behavior in intact, cycling female rats.

435.10

LORDOSIS CAN BE ELICITED IN CHRONICALLY-DECEREBRATE RATS BY COMBINED LUMBOSACRAL AND VAGINOCERVICAL STIMULATION.
J. D. Rose and F. W. Flynn. Department of Psychology, University of Wyoming, Laramie, WY 82071.

Previous research has indicated that chronically-decerebrate, adult female rats are incapable of exhibiting lordosis in response to lumbosacral somatic stimuli. This finding suggested that the rat's brainstem-spinal unit couldn't mediate lordosis in the absence of a facilitatory forebrain influence. Since vaginocervical stimulation facilitates elicitation of lordosis by lumbosacral somatic stimuli, the present study investigated the possiblity that decerebrate rats could show lordosis to lumbosacral stimuli if vaginocervical stimulation was applied concurrently. Ovariectomized female rats were decerebrated at the midbrain-diencephalic junction in a two-stage procedure. One to two weeks after decerebration, the animals were tested for lordosis responses to manually-applied flank, rump and perineal pressure stimulation during concurrent probe-pressure on the cervix. Of 9 rats tested, 5 exhibited clear lordosis. Estrogen-progesterone administration was neither necessary for lordosis elicitation nor very effective in promoting the response in animals which failed to show lordosis without hormone treatment. These results show that the brainstem and spinal cord of the adult rat can mediate lordosis in the absence of forebrain neural influences. Supported by NIH grants NS13748 and NS24879.

435.11

IBOTENIC ACID OR 6-OHDA LESIONS OF THE VTA INHIBIT SEXUAL RECEPTIVITY IN HAMSTERS.
C.A. Frye* and J.F. DeBold. Dept. Psychology, Tufts University, Medford, MA 02155.

Progesterone (P) implants in the VTA facilitate receptivity in estrogen-primed hamsters, whereas electrolytic lesions to the VTA inhibit receptivity. Since electrolytic lesions are nonspecific, we assessed the ability of axon-sparing lesions of the VTA to inhibit P facilitated receptivity. 5 µg ibotenic acid was infused into the midline VTA. After one week these animals were given 10 µg EB and two days later either 10 or 100 µg P. Ibotenic acid lesions of the VTA substantially inhibited receptivity induced by EB and 100 µg P. Neither controls nor experimentals responded to 10 µg P.

Because the VTA includes the A10 dopamine (DA) cell group we also examined the effects of DA selective lesions on receptivity. 7.5 µg 6-OHDA was infused into the VTA, with desmethylimipramine given so 30 minutes prior. 6-OHDA treatment localized partially or entirely in the VTA reduced the facilitative affects of 100 µg P on the total lordosis duration. After 100 µg P the controls had longer mean lordotic bouts than the 6-OHDA animals.

These results with axon-sparing lesions indicate that damage to fibers of passage do not explain our earlier results with electrolytic lesions and further strengthen our hypothesis that the VTA is an important region in the control of sexual receptivity in hamsters.

435.12

MORPHINE INFLUENCES ON ESTROUS BEHAVIOR AND MONOAMINE RELEASE FROM THE VENTROMEDIAL HYPOTHALAMUS.
I. Vathy and A.M. Etgen. Depts. Psychiatry and Neuroscience, Albert Einstein College of Medicine, Bronx, NY 10461.

It is well known that morphine (M) inhibits estrogen and progesterone-dependent female sexual behavior. M has also been shown to depress norepinephrine (NE) release in several brain regions. Our previous study employing microdialysis of the ventromedial hypothalamus (VMH) of freely moving rats demonstrated a substantial increase in NE release when females were actively engaged in mating behavior. Thus the present experiments used microdialysis to test the hypothesis that M inhibition of estrous behavior is associated with depressed NE release from the VMH. Monoamine levels in dialysates from awake, behaving animals were monitored throughout hormone priming (3 ug estradiol benzoate followed 44 hr later by 200 ug progesterone), M administration (10 mg/kg concurrently with progesterone) and behavioral testing. All animals were tested for estrous behavior with stimulus male rats beginning 4 hr after progesterone injection. As expected, reproductive behavior in hormone-treated females was associated with increased NE release from the VMH. M treatment was associated with inhibition of both estrous behavior and NE release from the VMH. Additional experiments will assess whether the opiate antagonist naloxone, which reverses M inhibition of sexual behavior, also reverses M suppression of NE release from the VMH. Supported by MH41414, NRSA MH15788, and RSDA MH00636.

435.13

CHANGES IN OXYTOCIN RECEPTOR BINDING ARE CORRELATED WITH CYCLIC FLUCTUATIONS IN OVARIAN HORMONE LEVELS. S. Pliskin*, A.E.Johnson and G.F. Ball (SPON: M. Numan). Section on Comparative Studies of Brain and Behavior, Laboratory of Clinical Sciences, NIMH, Poolesville, MD 20837 and Department of Psychology, Boston College, Chestnut Hill, MA 02167.

Oxytocin (OT) transmission is involved in the regulation of steroid-dependent sexual receptivity in the rat. Based on the results of hormone replacement studies, it has been suggested that 17ß-estradiol (E_2) sensitizes the brain to OT by increasing OT receptor binding in certain steroid-concentrating brain regions involved in the regulation of reproduction. In one of these brain regions, the ventromedial hypothalamic nucleus (VMN), dramatic changes in OT receptor binding occur after E_2 replacement. Furthermore, changes in binding occur within a time-frame relevant to steroid fluctuations characteristic of the estrous cycle. The purpose of these studies was to determine if steroid-dependent changes in OT receptor binding in the VMN occur in intact, cycling animals.

Adult female rats were exposed to sexually vigorous males and sexual receptivity was assessed. Animals were killed on the day in which sexual receptivity was observed (proestus) and 24 h (estrus) or 48 h (diestrus) later (N=4/group). Animals were killed by decapitation, blood was collected for plasma steroid assays and brains were removed and frozen on dry ice. Brain slices (20µm) through the VMN were cut on a cryostat and labeled with 50pM ^{125}I-d(CH_2)$_5$[Tyr(Me)^2,Thr^4,Tyr-NH_2^9]OVT (^{125}I-OVTA). Unlabeled OT (5µM) was used to define nonspecific binding. Receptor binding was measured using standard receptor autoradiographic methods (Kuhar, 1986) and plasma steroids were measured by RIA (Abraham, 1973).

Results of these studies showed that the highest levels of ^{125}I-OVTA binding in the vlVMN occurred during proestrous and early estrous with significantly lower binding values obtained in diestrus animals. Changes in OT receptor binding were correlated with fluctuations in circulating steroid levels. In agreement with other studies, high levels of E_2 and Progesterone were found in proestrous animals, with intermediate levels occurring late in diestrus and low levels during estrus. This study demonstrates changes in VMN OT receptors occur during the estrous cycle and may be involved in the regulation of steroid-dependent reproductive behavior.

435.14

CNS OXYTOCIN INFLUENCES SEXUAL AND SOCIAL BEHAVIOR IN PRAIRIE VOLES. D. M. Witt*, T. R. Insel and C. S. Carter*. (SPON: W. M. Saidel) Laboratory of Clinical Science, NIMH, Poolesville, MD 20837 and University of Maryland, Department of Zoology, College Park, MD 20742.

Sexual behavior is a potent stimulus for oxytocin (OT) release and centrally administered OT enhances sexual receptivity in rats. Unlike rats, female prairie voles are monogamous, reflex ovulators in which the first estrus is induced by male chemosignals. Durations and frequencies of postpartum matings (concurrent with lactation) are reduced significantly compared to mating obsserved during the male-induced estrus. It is hypothesized that OT released during birth, lactation, and sexual activity may inhibit female sexual behavior in prairie voles. Ovariectomized females (pretreated with estradiol benzoate) in confirmed estrus or non-receptive (oil-pretreated) females received OT in either intracerebroventricular (icv: 0.001, 0.3, or 1 µg OT or saline) or intraperitoneal injections (ip: 1, 3, or 10 µg OT or saline). There was a dose-dependent decline in the occurrence of lordosis following icv, but not ip, OT. Autogrooming increased following icv OT. Males paired with OT-treated females increased autogrooming. In non-receptive females, ip OT increased side-by-side contact and reduced aggression. Results suggest that OT may regulate female sexual behavior and may facilitate social behaviors leading to sexual arousal or social bonding.

Neural effects of OT presumably depend upon OT receptor availability. In other species, central OT receptor expression is influenced by ovarian steroids. In the present study, in vitro receptor autoradiography was used to examine the estrogen dependent induction of OT receptors in brain areas implicated in sexual behavior. OT receptor binding was compared in estrous versus non-estrous females (intact and ovariectomized). OT receptors were concentrated in several forebrain regions distinct from those previously reported in rats. Preliminary analysis indicates that exogenous or endogenous estrogen priming increased OT receptor binding in areas associated with olfaction, and in contrast to data from binding studies in rat brain, did not significantly increase in the ventromedial nucleus of the hypothalamus. Species differences in the neural activities of OT are suggested which may reflect an adaptive reproductive physiology and/or behavioral pattern.

LONG-TERM POTENTIATION IV

436.1

TOPOGRAPHICAL ORGANIZATION OF THE NIGROTHALAMIC PROJECTIONS IN THE RAT. I. Grofova Department of Anatomy, Michigan State University, East Lansing, MI 48824.

In the monkey, the major outflow of the substantia nigra pars reticulata (SNR) distributes to the motor thalamic nuclei (VAmc and VLm) which in turn project to the premotor and prefrontal cortices. However, in rodents, a corresponding connection is known to terminate exclusively in the ventromedial nucleus (VM) which gives rise to fibers terminating throughout the neocortex. We have previously reported that multiple iontophoretic injections of Phaseolus vulgaris (PHA-L) in the SNR labeled terminal fibers in the medial VL, dorsal zona incerta (ZI) and in the field of Forel (FF), in addition to the well known targets of nigrothalamic fibers (VM, PF, MD). The present report based on a series of single, small PHA-L injections in the SNR demonstrates that there exists a distinct topography of SNR projections. Fibers originating from the dorsal region of SNR distribute only to the VM, PF and MD while SNR cells in the ventral zone project also upon the medial VL, ZI, FF and the red nucleus. The dense terminal plexus of nigral fibers in VL has a patchy appearance, and the electron microscopic examination revealed that a significant proportion of the labeled terminals contact cell bodies and large dendrites.

These observations suggest that the portion of the rodent VL which receives nigral input may correspond to primate VAmc. Furthermore, it is notable that only the ventral zone of the SNR is related to structures which are associated with motor functions.

(Supported by N.I.H. grant NS25744).

436.2

KINDLING-INDUCED HIPPOCAMPAL LONG-TERM POTENTIATION (LTP) IS NOT BLOCKED BY MK-801. G. B. Robinson. Univ. of New Brunswick, Fredericton, N.B. Canada, E3B 6E4.

Recent evidence has raised the possibility that a blockade of LTP may contribute to the effectiveness of MK-801 in inhibiting the development of kindled seizures, (Abraham et al., Brain Res., 462, 1988; Gilbert, Brain Res., 463, 1988; McNamara et al., Neuropharmacol., 27, 1988). To test this hypothesis, New Zealand rabbits, deeply anesthetized with halothane, were implanted with stimulation and recording electrodes in the perforant path (PP) and granule cell (GC) layer of the dentate gyrus, respectively. Following surgery, rabbits received penicillin then were allowed 2 weeks to recover. For three days prior to kindling, the GC response to 10 PP impulses, applied at each of 10 intensities, was determined. On the fourth day, MK-801 (0.5 mg/kg, i.p) was injected and 2 hours later kindling stimulation was applied to the PP.

Significant LTP of the PP-GC response occurred following kindling. The magnitude of LTP was greater at 24 hours than at 30 minutes post-kindling. The additional increase in LTP magnitude, 24 hours after the kindling stimulation, may, in part, result from a masking effect of MK-801 (i.e., suppression of the PP-GC population spike; Abraham et al., Brain Res., 462, 1988). Supported by NSERC.

436.3

BLOCKADE OF LTP, KINDLING, AND KINDLING-INDUCED POTENTIATION BY THE NMDA-ANTAGONIST, MK-801. M.E. Gilbert and C.M. Mack. NSI Environmental Sciences, RTP, NC, 27709.

Antagonists of the NMDA-receptor subtype of glutamate delay the development of kindling and block longterm potentiation (LTP). It has been suggested that these antagonists may retard kindling by reducing seizure spread through their blockade of LTP. The present set of experiments tested 1) the efficacy of the NMDA antagonist, MK-801, to block LTP of the perforant path (PP) to dentate gyrus (DG) synapse in the unanesthetized rat; 2) the ability of MK-801 to retard PP kindling; and 3) the effects of MK-801 on synaptic potentiation induced by kindling afterdischarges (AD). Input/output (I/O) functions of responses evoked in the DG by single pulse stimulation of the PP were monitored before and 24 hr after LTP trains (10 50 ms, 400 Hz trains, 0.1 ms pulse duration at 1000 ua). Trains were delivered 30 min following 0, 0.1 or 1.0 mg/kg MK-801. Both dosages of MK-801 blocked potentiation of the population spike 24 hr post train delivery. Kindling was induced by daily delivery of stimulus trains (2 s, 60 Hz, 1.0 pulse duration, 800 ua) to the PP 30 min following 0 or 1.0 mg/kg MK-801. MK-801 increased AD thresholds, AD durations, and the number of sessions required to reach Stage 5 seizures. I/O functions monitored before kindling, and 24 hr after the 1st, 4th, 7th, 10th and 13th AD indicated that MK-801 completely blocked kindling-induced potentiation of the EPSP and reduced the enhancement of the population spike relative to control kindled subjects. These data are consistent with the notion that LTP contributes to the development of kindling.

436.4

APV AND URETHANE ANESTHESIA ALLOW PHARMACOLOGICAL DISSOCIATION BETWEEN THE MECHANISMS OF LTP AND KINDLING. D.P. Cain, F. Boon and E.L. Hargreaves. Dept. of Psychology, Univ. of Western Ontario, London, Ontario. CANADA N6A 5C2.

Some authors have suggested that long term potentiation (LTP) may be a major component of the mechanism of kindling. If this is so treatments that affect LTP should affect kindling similarly and vice versa. We tested this by administering APV and urethane to different groups of rats and subjecting them to LTP or partial kindling of the perforant path-dentate circuit, or both. Afterdischarge(AD)-evoked potentiation was also measured.

Urethane did not block potentiation of the EPSP or population spike (PS), but it completely blocked our ability to evoke AD using normal stimulation currents. In another group of rats urethane markedly raised the AD threshold and attenuated the duration of AD and prevented the increase in AD duration when AD was evoked at hourly intervals. It also blocked potentiation of the EPSP and PS due to the evoked AD.

In a third group of rats APV completely blocked all LTP effects, even when LTP stimulation three times the normal level was used. However, APV did not block increases in the duration of AD after repeated ADs, and it did not block potentiation of the EPSP or PS due to the AD.

The differences between the effects of these compounds in LTP and kindling suggests that the mechanisms of the two models may be substantially different. Supported by NSERC.

436.5

MODULATION OF HIPPOCAMPAL PRIMED BURST POTENTIATION BY ADRENALECTOMY AND CORTICOSTERONE. M.C. Bennett, D.M. Diamond, M. Fleshner and G.M. Rose. Dept. of Pharmacology, University of Colorado Health Sciences Center and VA Medical Center, Denver, CO 80262

Previously, we demonstrated that a low threshold form of long-term potentiation, termed primed burst potentiation (PB-LTP), is inhibited in behaving rats by novelty stress (Diamond et al., Neurosci. Abst., 477, 1988). This inhibition was not found in adrenalectomized (ADX) rats. Presently, we are studying the role of adrenal hormones as modulators of PB-LTP.

We recorded CA1 population spikes evoked by commissural stimulation in urethane anesthetized rats that were ADX-, sham-operated (SHAM), or ADX with a corticosterone pellet implant (ADX+CORT). In SHAMs, the magnitude of PB-LTP was negatively correlated with plasma CORT concentration. Further, the magnitude of PB-LTP was significantly greater in ADX than in SHAM subjects. Finally, in ADX+CORT subjects (which had a wide range of plasma CORT levels), the magnitude of PB-LTP was also related to the plasma CORT level: The magnitude of PB-LTP evoked in ADX+CORT rats which had low (non-stress) levels of CORT was greater than that found in ADX rats; the magnitude of PB-LTP evoked in ADX+CORT rats which had high CORT levels was reduced. These results suggest that CORT produces an inverted-U shaped modulation of hippocampal plasticity.

436.6

OPIOID ANTAGONIST ELIMINATES THE STRESS-INDUCED IMPAIRMENT OF LONG-TERM POTENTIATION (LTP). T. J. Shors, S. Levine, R. F. Thompson. Dept. Psych., Univ. Southern Calif., LA, CA, 90089 and Dept. Psychiatry, Stanford Univ. Stanford, CA 93405

In 1987, Foy et al. (Behav. Neural Biol. 48, 138) reported that behavioral stress impaired LTP in the rat hippocampus. Here we investigated a possible role for opioid peptides. Rats (n=10) were injected with 14 mg/kg naltrexone or saline (n=10), restrained, and exposed to 30, 1-sec tailshocks (1mA, 60 Hz). Ten controls were given naltrexone and 10 saline. Hippocampal slices (400um) were prepared. Field potentials were recorded from CA1 in response to tetanization of the Schaffer collaterals. Mean (± SEM) percent of baseline amplitude at 30 min. was: naltrexone/shock, 180% ±13; naltrexone, 157% ±8; saline/shock, 118% ±10; and saline, 186% ±12. ANOVA [$F(3,36)=7.90$, $P<0.01$] and Newman-Keuls indicated that the saline/shock group differed ($P<.0.05$) from the other three groups. Blocking peripheral and central opioid receptors prior to exposure to inescapable shock eliminated the stress-impairment in LTP, suggesting a modulatory role for opioid peptides. NICH&HD (HD02881), ONR (N00014-88-K-0112) and NIMH (MH11936).

436.7

VASOPRESSIN INVOLVED IN THE POTENTIATION OF TRANSMISSION IN THE LATERAL SEPTUM ELICITED BY BEHAVIOR. I.J.A. Urban, Y. Shen. G.Croiset, and A. Ontskul. Rudolf Magnus Institute, The University of Utrecht, The Netherlands.(SPON: ENA)
Foot shocks and/or training in conditioned avoidance task are thought to release the propresophysin (PPP) peptides vasopressin (VP), neurophysin II (NPII) and C-terminal glycopeptide CPP 1-39 in the lateral septum (LS). VP and CPP 1-39, but not PII, facilitate the glutamate(Glu)-ergic transmission between the fimbria (FI) fibers and LS neurons. With the negative wave (N-wave) of field potential (FPs), evoked by stimulation of the FI fibers, we examine effects of training in shuttle-box on this transmission in the rat LS of. Three series of 10 trials were administered in the acquisition and extinction sessions and the responses of the rats were recorded. The FPs recorded before and after the acquisition and extinction were compared. All Wistar rats classified as learners (6 or more correct responses in the last acquisition trial), showed 20-30% increase in the N-wave of the FPs after the acqiosition training. The N-wave before extinction was still on average 10% higher compared controls. After extinction, the N-wave increased again. The bad learners (less than 6 correct responses) showed only a 10-20% decrease in the FPs N-wave after both the acquisition and exctinction. The diabetes insipidus rats of the Brattleboro strain, either good or bad learners, showed only a decrease in the N-wave of the FPs after the acquisition and extinction. Thus, the training in shuttle-box can release principles in the LS that for hours facilitate the Glu-ergic transmission between the fimbria fibers and LS neurons. These principles might be PPP-like peptides.

436.8

LONG-TERM POTENTIATION OF EARLY AND LATE EXCITATORY POSTSYNAPTIC POTENTIALS IN KITTEN VISUAL CORTICAL CELLS. Y. Komatsu* and K. Toyama. Dept. of Physiology, Kyoto Prefectural Univ. of Med., Kamigyoku, Kyoto 602, Japan.

Synaptic transmission during and after conditioning stimulation (CS) of white matter (2 Hz, 15 min) was studied by intracellular recording from layer II-III cells in slice preparations of kitten visual cortex under perfusion with solution containing 1 μM bicuculline. The early (eEPSP) and late excitatory postsynaptic potentials (lEPSP) produced by white matter stimulation demonstrated the following characteristics. 1) The l- and eEPSPs were mediated by N-methyl-D-aspartate (NMDA) and non-NMDA receptors, respectively. 2) eEPSP was depressed during CS and potentiated after CS, while lEPSP was potentiated during and after CS. 3) Long-term potentiation (LTP) of e- and lEPSPs occurred together (7/13) or separately (1/13 for eEPSP, 3/13 for lEPSP) after strong CS. 4) Weak CS produced only LTP of lEPSP in a few cells (2/8), in which the lEPSP was potentiated during CS. 5) NMDA antagonists did not affect LTP of eEPSP while they blocked lEPSPs. These results suggest that the two LTPs are based on different mechanisms.

436.9

LONG-TERM POTENTIATION IN THE PREFRONTAL CORTEX FOLLOWING STIMULATION OF THE HIPPOCAMPAL CA1/SUBICULAR REGION. S. Laroche[1], T.M. Jay[2] and A.M. Thierry*[2]. [1]Dépt. de Psychophysiologie, CNRS, 91190 Gif-sur-Yvette, France and [2]INSERM U114, Collège de France, 75231 Paris, France.

Anatomical studies have provided evidence for a direct projection from the hippocampal/subicular region to the prefrontal cortex (PFC) in the rat (Jay et al., this meeting). Extracellular single unit recordings were obtained from 120 neurons in the prelimbic area of the PFC in 7 rats anaesthetized with pentobarbital. Fifty neurons (42%) exhibited excitatory responses to single pulse stimulation of the CA1/subicular region (single action potential, mean latency 18.3 ± 0.6 ms). Paired pulse facilitation was obtained for delays ranging from 40 to 200 ms in 11 of 20 neurons tested. Extracellular field potentials evoked by stimulation of the CA1/subicular region (0.033Hz) were recorded in the PFC in 7 other anaesthetized rats. The negative component of the field potential (peak latency 19.4 ± 0.54 ms) was measured off line. Two sets of 10 high-frequency (HF) trains (250Hz, 200ms) at 0.1Hz were applied at a 20-min interval and testing was then resumed for a period of one hour. Tetanic stimulation produced a significant and persistent potentiation of the amplitude of the negative component (63.94 ± 8.39 %) accompanied by a reduction of the onset latency. These results show that long-term potentiation can be induced *in vivo* in the rat prefrontal cortex by HF stimulation of the hippocampal CA1/subicular region.

436.10

FAILURE TO ERASE LONG-TERM POTENTIATION (LTP) BY COUPLING SUSTAINED PRESYNAPTIC ACTIVITY WITH NMDA CHANNEL BLOCKADE L.E. Chavez-Noriega*, R.S. Goldman and C.F. Stevens. Section of Molecular Neurobiology, Yale University School of Medicine, New Haven, Ct. 06510

A key feature of LTP as a model of information storage is the prediction of specific mechanisms for its reversal. Anti-Hebbian processes are likely candidates. We have studied the hypothesis that presynaptic activity in the absence of postsynaptic N-methyl-D-aspartate (NMDA) receptor activation should result in a reduction of synaptic efficacy.

Evoked field EPSPs were recorded in stratum radiatum of area CA1 of rat hippocampal slices before and after LTP induction, and then after sustained high frequency stimulation (SHFS; 2000-5000 pulses) in the presence or absence of the NMDA antagonist 2-amino-5-phosphonovalerate (APV). The change in EPSP slope recorded 30-45 minutes after SHFS in 50 uM APV was not different from that observed in slices stimulated in control medium. Any possible decrease in synaptic strength associated with presynaptic activity and blockade of the NMDA channel is well under 1% of the increase associated with LTP.

These results indicate that presynaptic activity uncoupled with NMDA receptor activation does not result in a long lasting decrease in synaptic efficacy.

436.11

UNEXPECTED PROPERTIES OF 'HEBB-SYNAPSES' IN RAT HIPPOCAMPAL SLICE CULTURES T. Bonhoeffer*, V. Staiger* and A.M.H.J. Aertsen* (SPON: V. Braitenberg). Max-Planck-Institute for Biological Cybernetics, Spemannstrasse 38, 7400 Tübingen, FRG.

In order to study the nature of synaptic enhancement in more detail we investigated whether hippocampal slice cultures (Gähwiler, B.H., *J. Neurosci. Meth.*, 4:329, 1981) display phenomena of synaptic plasticity. After we showed that phenomena like LTP do exist in this preparation, we used a paradigm developed by Gustafsson et al. (*J. Neurosci.*, 7:774, 1987) to enhance CA1-synapses: single presynaptic stimuli to the Schaffer-collaterals were paired with postsynaptic depolarizations using an intracellular electrode in the cell body of a CA1-neuron. Gustafsson et al. showed that this enhancement is spatially restricted on the postsynaptic dendrite: if one input to the postsynaptic neuron is strengthened, the strength of a neighboring input to the same neuron is not affected. In order to assess whether this observation also holds for the presynaptic axon (i.e. whether enhancement is also spatially restricted on the input fiber) we performed the following experiment. A stimulating electrode was placed in the afferent fibers, two closely adjacent CA1-cells (distance 25-60 µm) were impaled with intracellular electrodes and responses to a test stimulus were recorded from both of them. We enhanced synapses by pairing 30 single extracellular stimuli to the afferent fibers with depolarizing current pulses in one cell but not in the other cell. We examined the responses of both cells before, during and after the pairing. In all experiments in which we could induce synaptic enhancement in the 'paired' cell (n=7 out of 13), we observed that the responses of *both* cells were enhanced, although only one of them had received paired stimulation. This result was confirmed with optical recordings using voltage-sensitive dyes: over an area of some 150 µm around the paired cell *all* 124 recording sites showed significant enhancement.

These findings suggest that synaptic enhancement by the 'paired stimulation paradigm' is not strictly local on the presynaptic axons; even local postsynaptic stimulation results in '*synaptic recruitment*': the synapses of many neighboring postsynaptic cells are enhanced.

436.12

LOCAL BLOCKADE OF INHIBITION UNMASKS A CAPABILITY FOR LTP IN THE DENTATE COMMISSURAL PATHWAY THAT IS OTHERWISE NOT EXPRESSED. O. Steward, R. Tomasulo*, and W.B. Levy. Depts. of Neuroscience and Neurosurgery. Univ. of VA, Charlottesville, VA. 22908

LTP can be readily elicited in a number of hippocampal pathways, but is not expressed in the dentate commissural pathway. This pathway is similar to the commissural/ Schaffer collateral projection to CA1, except that its activation produces powerful inhibition that occurs nearly concurrently with the excitation. The present study evaluates whether this inhibition prevents the pathway from expressing LTP.

Acute neurophysiological experiments were carried out in urethane anesthetized rats. To locally block inhibition in the dentate gyrus, a recording micropipette filled with a solution of 8mM bicuculline in saline was positioned in the dentate gyrus. A control saline-filled micropipette was positioned nearby. The commissural pathway was activated by stimulating electrodes in the contralateral CA3/CA4 region. High frequency stimulation of the commissural pathway reliably elicited LTP at the bicuculline electrode, but not at the control electrode. This LTP required a threshold level of stimulation for its initiation. The high frequency stimuli induced an extracellular negativity at the bicuculline electrode that was not present at the control electrode. This negative potential was selectively blocked by ketamine and MK801, suggesting that the negative potential reflects NMDA receptor activation.

These results suggest that LTP is not normally expressed by the dentate commissural pathway because the simultaneous inhibition clamps the postsynaptic membrane at a potential that prevents the depolarization-related relief of Mg^{++} blockade of the NMDA receptor. Removal of inhibition would then relieve this blockade of NMDA receptor activation, enabling LTP. Supported by NSF Grant #BNS8818766 to OS and RCDA MH00622 to WBL.

436.13

PRECISE TEMPORAL INTERACTIONS DISTINGUISH INDUCTION OF LTP FROM LTD IN THE RAT DENTATE GYRUS. H. Hashemzadeh-Gargari & W.B. Levy. Dept Neurosurgery Box 420, UVA, Charlottesville VA 22908.

Levy and Steward ('83) showed that temporally staggered activation of the ipsilateral (IPS) and the converging contralateral (CNT) entorhinal cortex projections to the dentate gyrus results in either potentiation or depression of the CNT synaptic response. Depression results when CNT activation follows IPS activation; potentiation results when CNT activation precedes or is just simultaneous with IPS activation. These earlier experiments used 8 pulse (400 Hz) trains which gave a temporal resolution of ≥ 17.5 ms regarding the timing during conditioning. The present study used 3 pulse (400 Hz) trains in a similar staggered conditioning paradigm. Conditioning periods consisted of 16 of these very brief trains (1 train/ 200 ms). Four responses were measured: the IPS and CNT responses of both sides of the brain. The paradigm consisted of three conditioning periods with baseline response size established for 30 minutes before and after each conditioning period. In the first conditioning period the time between trains was 7.5 ms; in the second the time between trains was 2.5 ms. The last period also used 2.5 ms but reversed the IPS/CNT order (intertrain times are measured from the last pulse of the leading train to first pulse of trailing train). The statistically significant results with the 9 rats confirmed the ordering effect: CNT potentiation ensued when a CNT train lead an IPS train; with the reverse ordering CNT depression ensued in all cases (that is, whether or not prior potentiation or depression had been induced). The IPS responses potentiated. These results imply that the biochemical/biophysical reactions which determine whether potentiation or depression ensues from all but identical activation paradigms must account for differences of 7.5 ms or smaller. W. Holmes pointed out that there is a delay in NMDA receptor conductances which might account for this temporal sensitivity. Supported by NS15488 and NIMH RSDA K02-MH00622 to WBL.

436.14

SELECTIVE EFFECTS OF D-AMINO-5-PHOSPHONOVALERIC ACID (D-APV) ON MEDIAL AND LATERAL PERFORANT PATHWAY-EVOKED RESPONSES IN DENTATE GYRUS OF RAT HIPPOCAMPAL SLICE. D. Dahl and J.M. Sarvey. Uniformed Services University of the Health Sciences. Bethesda, MD 20814.

Differential effects of certain ligands have been obtained on granule cells to selective activation of the medial and lateral perforant pathways (PPs). Norepinephrine (NE; 1 µM), in the presence of phentolamine (50 µM), induces a potentiation of medial PP-evoked excitatory postsynaptic potentials (EPSPs) and population spikes and a concurrent depression of lateral PP-evoked responses. These effects are persistent. Induction of long-lasting pathway-specific modifications prompted us to examine N-methyl-D-aspartate (NMDA) activity in the dentate gyrus using the antagonist D-APV, which blocks LTP induction and NE-induced long-lasting potentiation.

A 15-30 min perfusion with a 10 µM concentration of D-APV had no effect on any component of extracellularly recorded EPSPs to selective stimulation of the lateral PP. D-APV was without effect on the initial slope of the medial PP-evoked EPSP, but the peak amplitude and late repolarization component of this response were depressed. No differential effect was found on medial and lateral PP-evoked population spikes with 10 µM D-APV: responses to activation of either pathway were depressed. All D-APV effects reversed to control levels upon wash with drug-free artificial cerebrospinal fluid.

These results indicate that D-APV differentially affects synaptic activation of granule cells via the PP. Components of medial PP-evoked EPSPs occurring at or following attainment of peak amplitude are depressed. The lateral PP-evoked EPSP is unaffected. Since both medial and lateral PP-population spikes were depressed by D-APV, a different mechanism from that affecting the EPSP may be involved. (Supported by NIH grant NS23865 to JMS.)

436.15

CA^{2+} INFLUX THROUGH NMDA-RECEPTOR CHANNELS--ITS ROLE IN LTP. INSIGHTS FROM BIOPHYSICAL MODELS. W.R. Holmes and W.B Levy. Math. Research Branch, NIDDK, NIH, Bethesda, MD 20892, and Dept. of Neurosurgery, U. Virginia Med. School, Charlottesville, VA 22908.

The induction of LTP is thought to depend on Ca^{2+} influx through NMDA-receptor channels. Ca^{2+} influx at a synapse on a dendritic spine and the resulting change in free Ca^{2+} concentration in the spine head were studied in a model of a hippocampal dentate granule cell as a function of input frequency and the number of co-activated afferents. These modeling studies provided a number of interesting insights. First, in order for the description of the NMDA-receptor mediated synaptic conductance used in the model to be consistent with previous experimental observations, the average number of open, unblocked NMDA-receptor channels on a single spine head at a given moment had to be very small (usually less than one). Second, no more than a four-fold change in Ca^{2+} influx was observed by increasing input frequency or by increasing the number of co-activated synapses (to 115 in this study). Third, relatively small increases in Ca^{2+} influx could produce extremely large increases in free Ca^{2+} concentration in the spine head. These increases in free Ca^{2+} concentration were highly dependent on and sensitive to the concentration and location of Ca^{2+} binding sites in the spine head. Thus, even though the nonlinearity of Ca^{2+} influx through NMDA-receptor channels due to increases in input frequency and the number of co-activated synapses is very important, it is not sufficient to explain associative LTP. In contrast, the control of free Ca^{2+} concentration in the spine head by Ca^{2+} buffering systems during and after Ca^{2+} influx may provide the required nonlinearity which must govern associative LTP. (This work was supported in part by NS15488 and NIMH RSDA K02-MH00622 to WBL.)

437.1

WIDESPREAD CORTICAL DENERVATION IN RATS FOLLOWING RECOVERY FROM THIAMINE DEFICIENCY R.G. Mair, A.E. Ferguson*, University of New Hampshire, R.L. Knoth, Chapman College, and P.J. Langlais, San Diego State Univ.

Rats recovered from a subacute bout of thiamine deficiency and pyrithiamine treatment provide a model of diencephalic amnesia characterized by impaired performance of behaviors requiring representational memory and the consistent occurrence of thalamic lesions, commonly restricted to the area of the internal medullary lamina. To discover the effects of these lesions on thalamocortical projections, a group of six Long-Evans rats were subjected to the anti-thiamine treatment, recovered for 1 to 2 weeks, sacrificed, and brain tissue processed for Fink-Heimer and cresyl violet staining of alternate serial sections through cortex. Signs of dense axonal degeneration were observed in layers 3 and 4 in areas of frontal, parietal, and occipital cortex. In individual animals patterns of denervation were bilaterally symmetric. These data suggest that the apparently limited lesions of this model of amnesia affect specific thalamic inputs in widespread areas of cortex.

437.2

IMPAIRED REVERSAL LEARNING BUT PRESERVED EXTRA-DIMENSIONAL SHIFT PERFORMANCE FOLLOWING N-METHYL-D-ASPARTATE (NMDA)-INDUCED LESIONS OF THE SUBSTANTIA INNOMINATA/VENTRAL PALLIDUM (SI/VP) IN THE MARMOSET. A.C.ROBERTS*, J.L.MUIR*, B.J.EVERITT*, T.W.ROBBINS. (SPON:W.WINLOW). Depts. of Expt. Psychology and Anatomy*, Univ. of Cambridge, Cambridge,UK.

Previous findings have indicated that NMDA-induced lesions of the SI/VP in marmosets lead to a reduction in ChAT activity in anterior cortex and to a range of behavioural deficits that may be related to frontal dysfunction. The present study investigated the effects of the same lesion on performance of a computerised task, analogous to a test sensitive to human frontal lobe damage, the Wisconsin Card Sort test. Animals received a series of compound visual discriminations varying along two abstract dimensions, one of which was differentially reinforced, followed by an extra-dimensional (ED) shift in which the previously irrelevant dimension was differentially reinforced. The lesioned marmosets showed an initial retention deficit, as seen previously, but gradual improvement over 3 successive discriminations and normal ED shift performance, as compared to controls. This preserved attentional shifting capacity is in contrast to the significant perseveration of responding on reversals of a visual discrimination following the same lesion, and suggests that the deficits resemble the effects of orbitofrontal and amygdaloid lesions, rather than lesions of dorsolateral prefrontal cortex.

437.3

QUISQUALATE-INDUCED LESIONS OF THE SUBSTANTIA INNOMINATA & VENTRAL PALLIDUM (SI/VP) IN THE RAT:EFFECTS ON ACQUISITION AND SERIAL REVERSAL OF A VISUAL DISCRIMINATION. T.W. Robbins, A.C.Roberts*, J.Muir*, R.Parker*, M.Gore* and B.J.Everitt*, (SPON:I.P. Stolerman). Depts. of Expt. Psychology and Anatomy*, Univ. of Cambridge, Cambridge,UK.

Lesions of the SI/VP using quisqualic acid can be employed to produce more specific reductions in cortical cholinergic markers than other excitotoxins. Such lesions fail to replicate behavioural deficits on a range of tests, calling for a re-evaluation of the behavioural effects of excitotoxic lesions of the cortical cholinergic projection. The present study investigated the effects of quisqualate-induced lesions of the SI/VP (which produced ChAT reductions of 50-60% in frontal and parietal cortex), on an operant, discrete trial discrimination reversal paradigm which is also sensitive to ibotenate lesions of the SI/VP. A comparable deficit was demonstrated in which the SI/VP lesioned rats performed as well as controls on a brightness discrimination but were impaired when the contingencies were reversed. Lesioned rats inhibited the previously correct response normally but were slower than controls to learn the response that had been previously incorrect. These results will be discussed in relation to the role of the cholinergic projections within their various target sites, especially the frontal cortex and amygdala, and compared to those found following lesions of the SI/VP in the marmoset.

437.4

EFFECTS OF NUCLEUS BASALIS LESIONS AND CORTICAL CHOLINERGIC GRAFTS ON ATTENTIONAL PERFORMANCE IN THE RAT. J.L. Muir*, S.B. Dunnett, B.J. Everitt*, T.W. Robbins. (SPON:S.B. Dunnett) Depts. of Experimental Psychology and Anatomy*, University of Cambridge, U.K.

There has been considerable emphasis on the role of forebrain cholinergic projections in learning and memory, but somewhat less on their attentional functions. Relatively specific destruction of cholinergic cells in the nucleus basalis of Meynert (nbM) was achieved by infusing quisqualic acid into the substantia innominata (SI). Cortical ChAT activity was reduced by 50% and there was no damage to the globus pallidus. These lesions impaired the rats' accuracy in localising brief visual targets in a 5-choice serial reaction time task. The deficit in choice accuracy was exacerbated by interpolating bursts of white noise. Latency to respond correctly and to collect food reward (magazine latency), were lengthened post-operatively and showed little recovery over several months. Preliminary results suggest that certain of the deficits (e.g. the disruptive effects of distracting white noise), were ameliorated by cholinergic-rich grafts placed in frontal cortical sites, whereas others (e.g. magazine latency), were not. These results suggest that the task deficits are attentional in nature and may be attributable to cholinergic deafferentation of the neocortex.

437.5

NEUROTOXIC LESIONS OF THE NUCLEUS BASALIS INDUCED BY COLCHICINE: EFFECTS ON SPATIAL NAVIGATION IN THE WATER MAZE. W. R. Mundy, S. Barone and H. A. Tilson. Lab. of Molec. and Integrative Neurosci., NIEHS/NIH, Research Triangle Park, NC 27709.

Previous studies have used excitotoxins such as kainic or ibotenic acid to examine the behavioral consequences of nucleus basalis (NB) lesions. In the present study, rats were given bilateral injections of colchicine (1.0 ug/site) into the NB and examined for changes in learning and memory. Unlike excitotoxins, which can produce extensive subcortical damage, colchicine produced a lesion limited to the site of injection. Histological studies demonstrated that colchicine decreased the number of choline acetyltransferase (ChAT) positive cells in the NB, and resulted in a marked loss of cortical acetylcholinesterase staining. Separate neurochemical analysis showed that colchicine lesions decreased ChAT activity in the neocortex but not the hippocampus or caudate nucleus. Rats with NB lesions showed a large deficit in a passive avoidance task, and were transiently impaired during acquisition of a reference memory task in the Morris water maze. In a reversal test in the water maze, the learning deficit reappeared. These data suggest that colchicine may be useful in producing specific lesions of the NB, which primarily affects the rate of acquisition of a spatial reference memory task.

437.6

PERINATAL CHOLINE SUPPLEMENTATION PRODUCES LONG-TERM MODIFICATIONS IN THE MORPHOLOGY AND DISTRIBUTION OF CELLS IN THE DIAGONAL BAND REGION EXHIBITING NGF RECEPTOR IMMUNOREACTIVITY. C.L. Williams, W.H. Meck, and R. Loy, Depts. of Psychol., Barnard Col. & Columbia Univ., New York, NY 10027 and Dept. of Surgery, Univ. of Rochester, Rochester, NY 14642.

Choline chloride supplementation during development has facilitative effects on spatial memory that extends well into adulthood (Meck et al., Dev. Psychobio. 21, 1988). We now report initial findings comparing the morphology and distribution of cells in the VDB that show NGF receptor immunoreactivity in perinatally choline treated rats and their controls. After behavioral testing, animals were sacrificed and horizontal sections through the diagonal band at 3 levels (bregma -6.6, -7.34 and -8.1 mm) were examined morphometrically. Perinatally choline-treated rats had somata that were larger, rounder, and had greater uniformity than controls and the caudal-rostral distribution of cells in the VDB was altered by perinatal choline treatment. These data suggest that perinatal choline supplementation may alter cholinergic function and improve spatial memory through enhancement of the NGF/NGFR system.

437.7

INCREASED WORKING MEMORY CAPACITY AS A FUNCTION OF PRE- AND/OR POSTNATAL CHOLINE ENRICHMENT: A CORRELATED ChAT IMMUNOCYTOCHEMISTRY AND GOLGI ANALYSIS IN THE DIAGONAL BAND AND DENTATE GYRUS.

Warren H. Meck and Christina L. Williams. Departments of Psychology, Columbia University and Barnard College, New York, New York 10027.

Choline chloride supplementation during embryonic days (ED) 12-16 and later during postnatal days (PD) 16-30 has been shown to produce long-lasting facilitation of spatial memory processes (Meck et al., J. Neurosci., in press). We now report that these two time frames represent anatomically and morphologically distinct phases of choline related influence. The choline effect that is produced during ED 12-16 is associated with increased cholinergic cell body clustering in the horizontal limb of the diagonal band. In contrast, the choline effect that is produced during PD 16-30 is dependent upon steroid induced sexual differentiation of the hippocampus and related brain areas during PD 1-15 and is associated with increased dendritic length and branching in the dentate gyrus. These time frame differences can be related to neurogenesis, neuronal death, and synaptogenesis in the basal forebrain and hippocampus, respectively.

437.8

FUNCTIONAL INTERACTION OF THE NUCLEUS BASALIS AND RAPHE DORSALIS IN SPATIAL MEMORY. P.J. Riekkinen Jr.*, J. Sirviö*, R. Miettinen*, A. Valjakka* and P.J. Riekkinen. Departments of Neurology and Pathology, University of Kuopio, Finland.

In the current study we compared the effects of an ibotenic acid lesion of the nucleus basalis (NB), a 5.7-dihydroxytryptamine lesion of the raphe dorsalis (RD) and a combined nucleus basalis and raphe dorsalis lesion in rats using water-maze test. RD lesion produced a marked and significant reduction of frontal (-73 %) and occipital (-71 %) cortex serotonin levels, but the hippocampal serotonin levels were non-significantly lowered (-10 %). NB lesion reduced choline acetyltransferase (ChAT) activity in the frontal (-35 %) and parieto-occipital cortex (-19 %), but hippocampal ChAT activities remained unaltered. NB lesioned animals were impaired compared to controls in both finding the submerged platform during training and remembering the previous location of it. Although RD lesion alone did not affect spatial learning in the water-maze, the RD lesion greatly potentiated the learning impairment produced by NB lesion. The group of rats with combined lesions showed longer escape latencies compared to NB lesioned rats, because they did not develop any place navigational strategies to locate the escape platform. In the group of rats with combined lesion the retention of the previous platform location also was further impaired compared to NB lesioned rats. The current results suggest a functional interaction between RD and NB nuclei in spatial learning. Furthermore they indicate that the serotonergic-cholinergic pathology may be importantly involved in the cognitive decline in Alzheimer's disease.

437.9

DISSOCIATION BETWEEN WATER MAZE ACQUISITION AND CHOLINERGIC DEFICITS FOLLOWING MEDIAL SEPTAL LESIONS WITH COLCHICINE. S. Barone Jr.[1,2], K. P. Nanry[1], and H. A. Tilson[1]. [1]LMIN, NIEHS/NIH, RTP, NC 27709 and [2] Dept. Anatomy and Cell Biol., ECU SOM, Greenville, NC 27858.

Colchicine (COL) was infused bilaterally into the lateral ventricles (3.75 ug/site) or directly into the medial septum (MS)(5 ug) of adult, male Fischer-344 rats and effects of behavior and cholinergic markers determined. Rats receiving intracerebroventricular (icv) administration of COL were hyperaggressive during the first wk after administration and were hyperactive when tested during 60 min sessions at weekly intervals during the first 3 wks after COL. Icv COL also interfered with the acquisition of a spatial task in the water maze. It was subsequently found that COL given icv decreased choline acetyltransferase (ChAT) immunoreactive cells in the MS and decreased ChAT enzyme activity in both the right and left hippocampus to about 50% of control levels. Rats receiving COL directly into the MS were also aggressive and hyperactive, but were not impaired in the acquisition of the water maze task. Decreased ChAT immunoreactive cell bodies in the MS and enzyme levels in the right and left hippocampus were observed in these animals. The results of these experiments do not support the conclusion that spatial learning deficits seen in animals with MS lesions are soley due to a lesion-associated cholinergic deficit.

437.10

MEDIAL SEPTAL LESIONS IMPAIR WORKING-MEMORY ON BOTH SPATIAL AND NON-SPATIAL OPERANT DELAYED ALTERNATION IN RATS. R. Numan, J.R. Quaranta, Jr.*, P.J. Murphy* and J.W. Marlow*. Department of Psychology, Santa Clara University, Santa Clara, CA 95053.

The septo-hippocampal system regulates spatial behavior, memory, and response flexibility (Numan, R., Physiol. Psychol., 6:445, 1978). This experiment determined which of these functions is disrupted by medial septal (MS) lesions which impair operant delayed alternation (DA). Male hooded rats received either MS lesions or a control operation. Following recovery, they were reinforced for alternating left and right lever presses in an operant chamber. The effects of various delays (0, 10, and 20 sec), and exteroceptive cues were assessed.

MS lesions did not impair alternation performance at a 0-sec delay, but did produce severe impairments at 10 and 20-sec delays. An exteroceptive cue which reduced the spatial requirements of the task did not ameliorate this impairment. However, an exteroceptive cue which reduced the working-memory (WM) requirements of the task did ameliorate the MS lesion induced deficit on DA. While the MS lesioned rats also made more perseverative errors than the controls, statistically removing this influence from the data did not modify the results. These data suggest that MS lesions in rats impair DA by disrupting the general process of WM rather than spatial behavior or response flexibility.

437.11

FORNIX TRANSECTIONS DISRUPT THE ONTOGENY OF LATENT INHIBITION IN THE RAT. M.M. Nicolle*, C.C. Barry*, B. Veronesi, and M.E. Stanton. Dept. of Psychology, UNC-Chapel Hill, Chapel Hill, NC 27514 and Neurotox. Div., U.S. EPA, Research Triangle Park, NC 27711.

Three experiments examined the ontogeny of latent inhibition in a conditioned taste aversion paradigm. In the first experiment, 18-, 25-, and 32-day-old rats received a pairing of a Sanka solution with LiCl injection (.75% BW,.4 M) or physiological NaCl after either 4 preexposures to the taste or control treatment without taste preexposure. Interference with conditioning by taste preexposure (latent inhibition) was only evident on postnatal-day 32 (PND32). Experiment 2 established the parametric generality of our failure to find latent inhibition on PND18. In Experiment 3, sham or fornix lesions were performed on PND18 and latent inhibition was examined on PND32. Lesioned subjects exhibited equivalent conditioned taste aversions to the CS+ flavor but failed to show latent inhibition. Lesions were verified by histogical examination of the fornix and loss of AChE staining in the hippocampus.

Thus, latent inhibition of taste-aversion learning develops between PND18 and PND32 and this development is disrupted by fornix transection during infancy. These findings suggest a role for septohippocampal maturation in the ontogeny of latent inhibition.

437.12

ANTICHOLINERGIC TREATMENT MIMICS THE EFFECT OF HIPPOCAMPAL ABLATION ON THE HOMING BEHAVIOR OF PIGEONS. V.P. Bingman, L. Chaves*, J.T. Erichsen and J.R. Krebs*. Dept. of Psychology, Bowling Green Univ., Bowling Green, OH 43403.

A major source of hippocampal afferents in birds originates from the diagonal band of the medial septum. Relying on immunohistochemical techniques, choline acetyltransferase (ChAT)-like immunoreactivity was observed in fibers ascending to the hippocampal formation,as well as in terminal-like neuropil of the same region. Additionally, ChAT-positive cell bodies were observed in that region of the diagonal band where projection neurons to the hippocampus were identified. Taken together, these results indicate that like the mammalian hippocampus, the avian hippocampus is in receipt of a cholinergic projection from the diagonal band of the medial septum.

The possible importance of this projection in the regulation of avian spatial behavior was examined by intramuscular injection of the anticholinergic drug scopolamine into homing pigeons prior to their being released. Scopolamine treated homing pigeons successfully oriented homeward in a manner that did not differ from saline injected controls. Scopolamine treated birds, however, did take significantly more time to return home. This pattern of results is also seen in hippocampal-ablated pigeons, suggesting that anticholinergic treatment and hippocampal ablation produce similar cognitive spatial behavior deficits.

437.13

VISUAL REVERSAL-LEARNING DEFICITS AFTER THALAMIC LESIONS IN PIGEONS. L. Chaves* and W. Hodos, Dept. of Psychology, Univ. of Maryland, College Park, MD 20742.

Shimizu and Hodos (Behav. Neurosci., 1989) reported that lesions of two laminae of the visual wulst (IHA and HD), both targets of the thalamofugal pathway, resulted in increased errors in a color reversal-learning task. This finding suggested that the thalamofugal pathway might play a role in visual discrimination involving stimulus context changes.

In the present study, lesions of the OPT complex (the thalamic source of afferents to IHA and HD) were found to have no effect on reversal-learning performance. Instead, we found that damage to nucleus rotundus (the thalamic component of the tectofugal pathway) resulted in deficits that were far in excess of those that had been obtained after IHA and HD lesions. We suggest that the reversal-learning deficits after wulst lesions are not due to the wulst's connections with the thalamofugal pathway, but rather to its connections with the tectofugal pathway.

437.14

EFFECTS OF LESIONS OF THE CHICK FOREBRAIN ON ONE-TRIAL PASSIVE AVOIDANCE LEARNING. T.A. Patterson, D.B. Gilbert and S.P.R. Rose. Brain and Behaviour Research Group, Open University, Milton Keynes, MK76AA England.

Three distinct nuclei of the chick forebrain--the intermediate medial hyperstriatum ventrale (IMHV), lobus paraolfactorius (LPO), and paleostriatum augmentatum (PA)-- show metabolic, morphological, and neurophysiological changes following training of day-old chicks on a passive avoidance task. Several experiments examined the effects of lesions in the IMHV or the LPO on the ability of chicks to learn and retain the avoidance task.

Bilateral lesions placed 24 hr before training in the IMHV produced an impairment in avoidance responding tested three hours after training. Pre-training unilateral lesions in the left but not the right IMHV resulted in a similar impairment. Bilateral IMHV ablations, given either one or six hours post-training, did not impair retention. In contrast, pre-training bilateral lesions of the LPO were not amnestic. Bilateral lesions of the LPO given one hour post-training produced significant amnesia. We are currently examining the effects of post-training unilateral LPO lesions.

These results are consistent with other studies that have examined the effects of bilateral IMHV lesions on acquisition and extend these findings by demonstrating lateralization of acquisition involving the left IMHV. The results also suggest that the IMHV is not necessary post-training to retain the memory for the avoidance training. The results of the LPO lesions indicate that the LPO is not necessary for acquisition of the avoidance response, but may maintain the memory trace following training. Hypotheses to account for these results and indications of future research will be discussed.

Supported by NIMH 1F32 MH09626 and SERC Grant GRE 57413.

SYMPOSIA THURSDAY PM

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Symposium. NEUROPEPTIDE REGULATION OF REPRODUCTION. Paul E Micevych, UCLA (Chairperson); R.B. Simerly, Salk Inst.; C. Malsbury, Memorial Univ.; S.P. Kalra, Univ. of Florida; S. Ojeda, Oregon Reg. Primate Ctr.

The regulation of reproduction by the nervous system involves the integration of endocrine and sensory cues. In the CNS, this integration has been attributed to a sexually dimorphic, gonadal steroid sensitive-circuit that includes specific loci in the amygdala, hypothalamus and brainstem. This symposium will examine both the role of gonadal steroids in mediating the function of this circuit and also the regulation of steroidogenesis by peptidergic inputs to the ovary. Paul Micevych will correlate the accumulation of gonadal steroids in sP- and CCK-immunoreactive cells with the distribution, expression and receptors of these peptides. Also discussed will be with the effects these peptides have on steroid-initiated sexual behavior. Richard Simerly will describe the selective gonadal steroid regulation of prepro-CCK mRNA as well as the regulation of androgen and estrogen receptor mRNA's. Charles Malsbury will present evidence that gonadal steroids maintain sP- and CCK- like inputs to loci in the circuit and that sP in this reproductively relevant circuit acts to facilitate reproductive behaviors in male and female rats. Satya Kalra will discuss the regulation of gonadotropin secretion, the effects of gonadal steroids on NPY secretion, and the interaction with the endogenous opioid system. Sergio Ojeda will complete the presentations by elucidating functional roles for the VIP-ergic and sympathetic innervation of the ovary and their effects on the development of this organ.

440

SYMPOSIUM. INHIBITORY INFLUENCES ON GROWTH CONES AND CELLS. M.E. Schwab, Univ. of Zurich (Chairperson); J. Raper, Univ. of Pennsylvania; F. Bonhoeffer*, Max Planck Inst. for Devel. Biol., FRG; R. Chiquet-Ehrismann*, Friedrich Miescher Inst., Switzerland; S.B. Kater, Collorado State Univ.

Substrates favoring neurite growth as well as outgrowth promoting and chemotactic soluble factors are crucially involved in nervous system development. Recently, however, several lines of evidence point to the existence of antagonistic, inhibitory mechanisms. Non-permissive substrate properties restricting neurite growth or cell migration can be associated with cell surfaces or ECM components. In addition, specific neurotransmitters can arrest growth cone mobility.

Inhibitory components were found in membranes of neurites of specific subsets of neurons (J. Raper), in a regionalized pattern in the embryonic chicken tectum (F. Bonhoeffer), and on oligodendrocytes and CNS myelin (M.E. Schwab). For tenascin, a non-permissive substrate found in the ECM of the developing PNS and CNS, the molecular analysis reveals a domain structure and leads to models for the mechanism of action (R. Chiquet). The drastic inhibitory effect of neurotransmitters on growth cones seems to involve calcium-mediated mechanisms (S.B. Kater).

Thus, neurite growth and tract formation in the developing NS may be crucially influenced by inhibitory mechanisms at various stages of development.

VISUAL CORTEX VI

441.1

PRIMARY VISUAL CORTEX IN MACAQUE IS A CONFORMAL MAP OF ITS TRUE SENSORY SURFACE, A COMPOSITE OF THE TWO RETINAS. Stanley J. Schein and Pablo Lapuerta*. Howe Laboratory, Mass. Eye & Ear Infirmary, Harvard Medical School, Boston, MA 02114.

Schwartz (1977) proposed that the mapping of the visual field surface to the surface of V1 was conformal. This elegant and powerful mathematical description requires isotropy, that linear cortical magnification at any point in V1 be independent of direction. Recent published data (Tootell et al) is not consistent with isotropy, a finding that may be related to replication of the visual field in adjacent ocular dominance (OD) stripes.

Our new mathematical analysis, applied to the recent data, shows that V1 may indeed be a conformal map, but only if what is mapped is a composite of 2 surfaces, the 2 retinas. The OD stripe system in V1, then, reveals how each retina is "sliced" before being interleaved to form the composite retina. The degree of local anisotropy in the map reveals how retinal slices are stacked to form the composite. The pattern of slicing and the method of stacking may be measured independently.

This analysis also provides a formal mathematical method for back-mapping V1 onto the retina. Contrary to previous reports, we find that OD stripes may back-map to horizontal "slices" in the visual field. Computation of binocular visual functions may take advantage of the horizontal arrangement of OD stripes. Conversely, the arrangement of the OD stripes could be configured postnatally by circuits that promote stripe formation along the direction of greatest disparity, that is, the horizontal.

441.2

NONINVASIVE COMPUTATIONAL CARTOGRAPHY OF HUMAN VISUAL CORTEX BASED ON MAGNETIC RESONANCE IMAGING (MRI) AND POSITRON EMISSION TOMOGRAPHY (PET). G.J. Carman and B.N. Mora*. Division of Biology, Caltech, Pasadena, CA 91125.

The development of algorithms for the production of unfolded maps of the cerebral cortex (1) has made it possible to map extensive areas of the human cortex for the first time. Here we report the application of these techniques to data obtained from MRI and PET scans of the brain of a single human subject so as to yield a mapping of cerebral blood flow (CBF) as a function of location on an unfolded map of the cortex. Contours delineating the surface of cortex were digitized from MRI scans and used to compute a three-dimensional reconstruction of the cortical surface through application of differential geometry. Since the cortical surface is intrinsically two-dimensional, it can be "unfolded" by computing a mapping of the reconstruction onto the plane. An approximately conformal mapping, in which measures of length and angle are preserved with an absolute minimum of distortion, can be obtained through application of the optimization technique known as "simulated annealing" (1). Once this mapping is obtained, PET data in register with the MRI data can be projected onto the unfolded cortex so as to yield a map of differential CBF as a function of cortical location arising from controlled visual stimulation.

(1) Carman, G.J. and Van Essen, D.C. Soc. Neurosci. Abstr. 11:1243, 1985.

441.3

THE MORPHOLOGY OF PRIMATE W-LIKE GENICULOCORTICAL AXONS WHICH ARBORIZE IN AREA 17. *E.A. Lachica* and V.A. Casagrande*. Dept. Cell Biology, Vanderbilt Univ. Sch. Med., Nashville, TN 37232 (SPON: J. Hutchins).

Previous studies have shown the lateral geniculate nucleus (LGN) of the prosimian primate, *Galago*, to be composed of three pairs of layers comprised of magnocellular (Y-like), parvocellular (X-like) and koniocellular (W-like) cells. In the present study, we injected PHA-L into the W-like LGN layers, reconstructed the arbors that terminated in striate cortex (area 17) then compared the location of these arbors to the patterns of cytochrome oxidase (CO) on alternately stained sections. The results show that all W-like arbors terminate within individual CO blobs in layer III of area 17. Many of these axons bifurcate in layers V or VI producing a collateral that arborizes in layer I. No axon possessed collaterals that terminated in more than one blob or in any other cortical layer. The W-like arbors are about the same size (0.027 $\pm$ 0.013 mm^2) as X-like arbors that terminate in layer IVβ. They are, however, smaller than Y-like arbors (0.08 $\pm$ 0.01mm^2) that terminate in layer IVα. While W-like arbors have fewer boutons than Y-like arbors, they have about the same number of boutons as X-like arbors. This suggests that the W-like pathway could provide significant input to the CO-blobs in these primates. (Supported by EY01778 to VAC & MH09754 to EAL).

441.4

LAYOUT OF ORIENTATION COLUMNS IN FERRET VISUAL CORTEX: A DOUBLE-LABEL 2-DEOXYGLUCOSE (2-DG) STUDY. C. Redies and M. Diksic*. Montreal Neurological Institute, Montreal, Quebec, Canada H3A 2B4.

The visual cortical organization in the orientational domain was studied in six NO_2/O_2 anesthetized ferrets using a simplified version of the quantitative double-label 2-DG technique (Redies and Diksic, *Neurosci.*, 1989). 5 mCi of [^{3}H]2-DG and 25 μCi of [^{14}C]2-DG were used as tracers. Stimulations lasted 60 and 30 min, respectively. Cortical activation patterns were separated in each animal using a subtraction procedure that corrects for uptake of the first tracer during the second stimulation (Redies et al., *Neurosci.* **22**: 601, 1987) and for cross-contamination between the isotopes.

Stimulation with two gratings of *orthogonal* orientation elicits activation patterns that are partially complementary and show little overlap between orientation columns. Patches not activated by either stimulation suggest a mosaic-like pattern with intermediate orientations represented in relatively large spaces between the orthogonal orientations. With orientations differing *by 45 degrees*, a complex pattern of partial overlap is observed. The orientational changes show frequent alterations in their magnitude and direction. No evidence of any modularity was found. The maps obtained in this study are strikingly similar to computer-generated maps simulating development of neural connectivity (Linsker, PNAS **83**:8779, 1986). Supported by MRC of Canada (MA-10233).

441.5

INFLUENCE OF A BINOCULAR CENTRAL RETINAL LESION ON THE GAD-IMMUNOREACTIVITY IN CAT VISUAL CORTEX AND dLGN. F. Vandesande, H. Demeulemeester*, G.A. Orban and U. Eysel. Lab. Neuroendocrinologie, Naamsestraat 59, B-3000 Leuven, Lab. Neuro- en Psychofysiologie, K.U.Leuven, Campus Gasthuisberg, B-3000 Leuven, Belgium and Dept. Neurophysiology, Ruhr-Universität Bochum, D-4630 Bochum, FRG.

Hendry and Jones (Nature, 320:750, 1986) reported that monocular deprivation in monkey results in a strong decrease of the number of ir-GABAergic neurons in the ocular dominance columns associated with the deprived eye. In the present study, we used a binocular paradigm in order to exclude the influence by the intact contralateral eye. Four normal and four experimental cats were involved. The latter received a central retinal lesion (diameter 10°) in both eyes. In such a paradigm each cat can serve as a control for itself. After four weeks survival the laminar distribution of ir-GAD neurons in area 17 and the dLGN was calculated and expressed as a percentage of the total neuronal population. In layer 2-6 of the cortex subserving central vision of experimental animals the proportion of GAD in cells was 3 to 5 times higher than that of control animals. In the cortex subserving peripheral vision the proportions were equal in experimental and control animals. In the part of the layer A and A_1 of the lateral geniculate nucleus subserving control vision the proportion of GAD in cells was reduced in experimental animals compared to control animals. These results show that the effect of visual stimulation of concentration of GAD in cortical cells is more complex than previously thought.

441.6

REGULATION OF CYTOCHROME OXIDASE PROTEIN LEVELS BY FUNCTIONAL ACTIVITY IN THE MACAQUE MONKEY VISUAL SYSTEM. R. F. Hevner and M. T. T. Wong-Riley. Dept. of Anatomy & Cellular Biology, Medical College of Wisconsin, Milwaukee, WI 53226.

Cytochrome oxidase (C.O.) is a mitochondrial energy metabolic enzyme used as a marker of neural functional activity. The histochemical activity of C.O. in the striate cortex (and in other brain regions) can increase or decrease in response to altered neural functional activity (Wong-Riley and Carroll, *Nature* **307**:262, 1984). Enzyme activity changes could be due to modulation of enzyme protein levels or turnover number. To determine if C.O. protein levels change with altered neural functional activity, we examined the distribution of C.O. protein immunohistochemically in the visual system of macaques treated for 3-4 weeks with monocular intravitreal injections of tetrodotoxin. The antiserum to C.O., and the immunohistochemical method, have been described previously (Hevner and Wong-Riley, *J. Neurosci.*, in press). In the striate cortex, as well as in the LGN and retina, changes in C.O. immunoreactivity were observed after tetrodotoxin treatment, and closely followed changes in C.O. activity. Ocular dominance banding was visible by both methods in most cortical layers, and was most pronounced in layer 4C. The results show that neuronal C.O. protein levels are regulated by neural functional activity. We propose that C.O. protein levels are constantly adjusted to meet neuronal energy metabolic requirements. (Supported by NIH NS18122 & EY05439 to MWR, and a MCW MSTP Fellowship to RFH.)

441.7

NEUROCHEMICAL PROPERTIES AND POSTSYNAPTIC TARGETS OF CHOLINERGIC SYNAPSES IN CAT VISUAL CORTEX. C. Beaulieu and P. Somogyi, MRC Anatomical Neuropharmacology Unit, South Parks Rd, Oxford, England.

GABA-content and synaptic connections of cholinergic terminals has been studied using combined immunocytochemistry for CHAT (pre-embedding) and GABA (post-embedding). To date, 101 CHAT(+) synapses were analyzed and of the identified postsynaptic targets, 18% were spines, 33% GABA(+) dendrites, 47% GABA(-) dendrites, and 1% GABA(-) somata. The proportion of cholinergic synapses contacting GABA(+) elements is very different from that found for the total population of synaptic contacts. From a sampling of 1061 synapses, only 8% of GABA(+) and 10% of GABA(-) boutons targeted GABAergic elements. This suggests that cholinergic terminals preferentially contact GABAergic cells; they terminate 3 times more frequently on GABAergic targets than the overall population of synapses.

Furthermore, 14% of the CHAT(+) synaptic boutons analyzed were also GABA(+). The distribution of these double-labelled boutons on postsynaptic elements is similar to that obtained for CHAT(+) boutons which were GABA(-). The source of GABA-containing cholinergic terminals could be the basal forebrain or they could arise from local cells as in the cortex of rat. However, we have not found any CHAT(+) cell in the cat cortex.

441.8

EXCITATORY TRANSMITTER AMINO ACID-CONTAINING NEURONS AND THEIR PROJECTIONS IN THE RAT VISUAL CORTEX J.G. Parnavelas*, I. Dori* and A. Dinopoulos* (SPON: European Neuroscience Association). Dept. of Anatomy, University College London, London WC1E 6BT, U.K.

The distribution and morphology of neurons labeled immunocytochemically with antisera to glutamate (Glu) or aspartate (Asp) were examined in the rat visual cortex. Using well-established light microscopical features as well as nuclear, cytoplasmic and synaptic criteria, we noted that Glu-labeled neurons were exclusively pyramidal distributed in layers II-VI with increased concentration in layers II & III. Asp-immunoreactivity was localized chiefly in pyramidal neurons located in layers II-VI but also in a small percentage (approximately 10% of labeled cells) of nonpyramidal cells scattered throughout the cortical thickness. In order to examine the amino acid-containing neurons in the visual cortex which give rise to various corticofugal and calloral projections, we utilized immunohistochemistry in conjunction with HRP histochemistry. The results of this study showed that Glu and Asp are the putative transmitters in substantially different proportions of neurons in the efferent systems examined suggesting a chemical heterogeneity of these systems.

441.9

DIRECT DEMONSTRATION OF THE RELATIONSHIP BETWEEN INTRINSIC AND EXTRINSIC CORTICO-CORTICAL CONNECTIONS USING DOUBLE LABEL TRACT TRACING. R. Malach Center for Neurosciences, Weizmann Inst. of Science, Rehovot, Israel 76 100.

The relationship between the intrinsic (within a cortical area) and extrinsic (between cortical areas) horizontal connections was studied using double label ,tract tracing approach. Single injections of the fluorescent tracer bisBenzimide were placed in cat area 18. The resulting label in area 17 consisted of a set of banded patches which were revealed in the whole, fixed, brain preparation. Under visual guidance, a crystal of the tracer DiI was inserted into one of the BB-labelled patches. Following 2 months of incubation, the DiI produced intense fiber labelling, which, in tangential sections, appeared to radiate from the injection site, forming several tongues and patches. Comparing the BB labelled extrinsic patches and the DiI labelled intrinsic connections revealed clear instances of overlap but no cases of interdigitation in the material studied . The applicability of the double tracer approach for revealing the relationship between intrinsic and extrinsic connections in different cortical areas and different mammalian species will be assessed.

Supported by grants:BSF 85-00258,Inst. Psychobiol.

441.10

MODULAR CONNECTIONS OF EXTRASTRIATE VISUAL AREA DM WITH AREAS 17, 18, AND MT IN MONKEYS. L.A. Krubitzer and J.H. Kaas, Vanderbilt University, Nashville, Tenn.

Injections of WGA-HRP in the dorsomedial visual area, DM (V3?) of owl monkeys (Aotus trivirgatus) labeled arrays of clusters of neurons in area 17 and rows of neurons crossing much of the width of area 18 (V-II). In addition, rows of clusters of neurons and terminals were labeled in the middle temporal visual area, MT. Thus, restricted locations in DM appear to relay information from a subset of neurons in area 17 and bands of neurons in area 18 to several aligned rows of neurons in MT. Inputs from aread 17 and 18 were from regions including both the representationd of the upper and lower visual quadrants, and microelectrode mapping experiments confirmed that DM, unlike V3d, represents the upper as well as the lower visual quadrant. Other connections of DM were with part of posterior parietal cortex and MT projection fields ST and FST. The pattern of connections indicates that DM is part of the "magnocellular" stream of cortical processing related to functions of the parietal lobe such as visual attention and localization. (EY-02686)

441.11

CORTICAL CONNECTIONS OF INFERIOR TEMPORAL CORTEX IN SQUIRREL MONKEYS. R. E. Weller, J. F. Hood* and G. E. Steele. Dept. of Psychology, Univ. of Alabama at Birmingham, Birmingham, AL 35294.

All primates appear to have a large region of inferior temporal (IT) cortex that is visual in function. We determined the connections of caudal IT cortex in squirrel monkeys (Saimiri sciureus) by making injections of neuroanatomical tracers in different locations in caudal IT. Tracers such as the fluorescent dyes Fast Blue and Diamidino Yellow, and wheat germ agglutinated-horseradish peroxidase were injected under sterile conditions in anesthetized animals. After appropriate survival times, animals were sacrificed and the brains frozen, sectioned and processed histologically. Major input to IT cortex originated from the caudal part of the Dorsolateral Area, DLc. Weaker projections came from rostral DL, MT, lateral V II, and locations in the frontal lobe. Injections in central, caudal IT cortex also revealed connections with other parts of IT cortex, such as more rostral IT, dorsal IT in the superior temporal sulcus, and ventral IT in the inferior temporal sulcus and medial to the inferior temporal sulcus. These intra-IT connnections suggest the existence of subdivisions within IT cortex of squirrel monkeys.

Supported by NIH grants R29 EY07147 to R.W. and T32 EY07033 to G.S.

441.12

ORGANIZATION AND CONNECTIONS OF THE CAUDAL DORSOLATERAL AREA IN SQUIRREL MONKEYS. G. E. Steele, C. G. Cusick and R. E. Weller. Dept. of Psychology, Univ. of Alabama at Birmingham, Birmingham, AL 35294 and Dept. of Anatomy, Tulane Univ., New Orleans, LA 70112.

In monkeys, visual information reaches inferior temporal (IT) cortex via projections from an area of cortex called V4 in macaque monkeys and the Dorsolateral Area (DL) in owl monkeys. V4 extends from lateral to ventral cortex, while DL is restricted to the dorsolateral surface. The present study examined which of these organizational schemes exists for cortex in the location of caudal DL (DLc) in the squirrel monkey, Saimiri sciureus. This was accomplished by dorso-ventrally varying the locations of injections of neuroanatomical tracers in cortex rostral to V II and examining the resulting patterns of connections. All but the most dorsal injections resulted in a topographic pattern of connections with V II, strongly suggesting that cortex from dorsolateral to ventral comprised a single area, the extent of which was similar to V4 in macaques. We also found evidence that DLc projected to caudal IT in at least a crudely topographic fashion, with stronger projections originating from the parts of DL representing central vision.

Supported by NIH grants R29 EY07147 to R.W. and T32 EY07033 to G.S.

BIOCHEMICAL AND PHARMACOLOGICAL CORRELATES OF DEVELOPMENT III

442.1

INDUCTION OF A PLACENTAL-LIKE ALKALINE PHOSPHATASE AND REDUCTION IN PROTEIN TYROSINE PHOSPHORYLATION CONCURRENT WITH BUTYRATE-INDUCED NEURONAL TRANSFORMATION OF THE TE671 CLONAL CELL LINE. Mario B. Marrero* and Ronald J. Lukas (SPON. H.N. Siegel). Division of Neurobiology, Barrow Neurological Institute, Phoenix, AZ 85013.

We have previously reported that cells of the TE671 human medulloblastoma clonal line exposed to the 'differentiating agent' sodium butyrate undergo morphological transformation to a state resembling that of mature neurons in a time- and concentration-dependent manner. These cellular morphological changes involving neurite extension, a 2-3-fold increase in alkaline phosphatase activity, and an induction of a placenta-like alkaline phosphatase isoenzyme as show by Western immunoblot procedures all are apparent following two-three days of treatment with 2 mM sodium butyrate. Western immunoblots probed with a monoclonal antibody against phosphotyrosine indicate that butyrate treatment induces a striking decrease in the phosphotyrosine content of a number of proteins, but that these effects occur within one hour of butyrate treatment. All of these effects are maintained for the duration of butyrate exposure. The results suggest that several mechanisms involving cellular protein phosphorylation/de-phosphorylation might be involved in butyrate-induced neuronal transformation of the TE671 clonal cell line.

442.3

CHARACTERIZATION AND PARTIAL PURIFICATION OF NEURON SPECIFIC GLYCOPROTEINS FROM *DROSOPHILA MELANOGASTER*. Muñoz-Maines, V.* and Salvaterra, P. (SPON: K. Ikeda). Division of Neurosciences, Beckman Research Institute of the City of Hope, Duarte, CA 91010.

Antibodies directed against the plant glycoprotein horse radish peroxidase (HRP) recognize an epitope expressed by all neurons at different stages of *Drosophila* nervous system development. The isolation and characterization of the specific neuronal antigen(s) bearing the anti-HRP epitope(s) could be a potentially valuable tool for studying neuronal development. We have used Western blots of *Drosophila* proteins, stained with anti-HRP antibodies, to characterize and partially purify the glycoproteins from 2nd instar larvae and adult fly heads.

Detergent extracts from larval stages show a single immunoreactive polypeptide with a molecular size of 52 kD. Immunostaining is no longer observed when the sample is oxidized with sodium periodate, indicating that the epitope recognized by the antibodies is a carbohydrate (i.e. a glycoprotein). This glycoprotein appears to be heterogeneous with respect to its carbohydrate composition. Purification of the antigen from second instar larvae, using detergent extraction, anion exchange chromatography and preparative gel electrophoresis results in a 30% pure sample of antigen which is being used to generate monoclonal antibodies to the protein core.

In contrast to larval stages, a homogenate from adult fly heads shows 2 immunostained polypeptides of 43 and 70 kD. Both bands are at least partially aqueous soluble. The anti-HRP staining of both bands is sensitive to periodate oxidation.

442.3

MOLECULAR MARKERS OF THE MOTOR AND MECHANOSENSORY NEURONS IN THE NEMATODE CAENORHABDITIS ELEGANS. S. S. Siddiqui and H. Yasuda *. Laboratory of Molecular Biology, Toyohashi University of Technology, Toyohashi 440, JAPAN.

We have previously described the staining pattern of a number of anti-tubulin monoclonal antibodies to the neural and non-neural cells of the nematode Caenorhabditis elegans. The antibodies also recognize specific tubulin isotypes from C. elegans separated by isoeclectric focusing on gels using ampholines(Siddiqui, S.S., Aamodt, E., Rastinejad, F., and Culotti, J. J. Neurosci, 1989 in press). Among the antibodies, 6-11B-1, raised against acetylated alpha tubulin, preferentially stains the six mechanosensory neurons, and 2-28-33, stains the inhibitory motor neurons VD and DD in the ventral cord of the nematode. Another antibody E6B6 is specific to beta tubulin, and stains the entire nervous system. Here we have screened a lambda gt11 library of C. elegans cDNA to screen the expression library with the tubulin antibodies. We have isolated positive clones using the E6B6 antibody, and two other alpha tubulin antibodies 3A5, and 4-1-B6, subcloned in pUC118 vector, and sequenced. The SQ#TbB03 clone shows a partial sequence of a beta tubulin, whereas, SQ# TA01, has a sequence of an alpha tubulin. This alpha tubulin gene is more homologous to a human alpha tubulin, then it is homologous to the yeast, or Drosophila melanogaster alpha tubulin gene. The sequence analysis of the beta tubulin gene is in progress, and the data for its cellular localization will be presented.

442.4

ENVIRONMENTAL ENRICHMENT RESULTS IN ENHANCED ACTIVITY OF HIPPOCAMPAL SUBICULUM AND VISUAL CORTEX IN YOUNG RATS: A 2-DEOXYGLUCOSE STUDY. E.M. Gonzalez-Lima*, P.A. Ferchmin, V.A. Eterovic and F. Gonzalez-Lima. Dept. Med. Anat., Texas A&M Univ., College Station, TX 77843, and Dept. Biochem., Sch. Med., Univ. Central del Caribe, Cayey, PR.

The goal was to determine functional changes in the rat brain resulting from 2 types of housing. Male, Tryon S1 rats were weaned and kept together for 4 days. Then pairs matched by weight were randomly assigned to: 1) rats housed individually in standard lab cages as the "impoverished" condition (IC), or 2) rats in the "enriched" condition (EC) in a complex environment of 2 large cages with inanimate objects and conspecifics. After 4 days, rats were injected with 2-DG, placed individually in a standard lab cage and killed after 90 min. Brains of EC rats were significantly heavier (57 mg) than IC, but no differences in body weights were found. EC brains showed significantly enhanced 2-DG uptake in the hippocampal subiculum (36%) and visual cortex (27%). There was also a 10% increase in thickness in the visual cortex but not a similar change in the subiculum. No changes were observed in other cortical areas (frontal, parietal, temporal, cingulate, piriform) or in the midbrain reticular formation. 2-DG changes in EC brains are consistent with activation of structures related to visual learning rather than nonspecific arousal. The findings provide a neural basis for the improved learning abilities shown by EC rats. (R01MH43353, NIH-RCMI)

442.5

DEVELOPMENT OF CYCLIC AMP-DEPENDENT PROTEIN KINASE SUBUNIT MESSENGER RNA LEVELS IN THE FETAL RAT BRAIN. J.S. Massa*, R.A. Maurer* & R.E. Fellows. Dept. of Physiology and Biophysics, Univ. of Iowa, Iowa City, Iowa 52242.

cAMP in the brain acts via cAMP-dependent protein kinase (cAMPdPK). Using rat cDNAs for all known isoforms of cAMPdPK regulatory (R) and catalytic (C) subunits (RIα, RIβ, RIIα, RIIβ, Cα, Cβ) we observe that, in the fetal rat brain from E12 to birth, while α subunit (RIα, RIIα, Cα) mRNA levels are abundant and relatively constant, β subunit (RIβ, RIIβ, Cβ) mRNA levels increase from undetectable or very low levels to abundant levels. Furthermore, while α subunit mRNAs are abundant in both primary neuronal and primary glial cultures, β subunit mRNA levels are very low (Cβ) or undetectable (RIβ, RIIβ) in primary glial cultures, but are abundant in primary neuronal cultures. Thus, prior to about E12, cAMP may act only via the α cAMPdPK subunits in neuronal and glial precursor cells. After about E12, the β set of cAMPdPK subunits may also mediate the effects of cAMP in neurons. [Supported by NIH GM 35903 (to RAM), NS 24629 (to REF) and Univ. of Iowa Diabetes and Endocrinology Research Center Grant DK 25295.]

442.6

DEVELOPMENTAL CHANGES IN NMDA RECEPTOR FUNCTION STUDIED IN A GREASE-GAP PREPARATION OF HIPPOCAMPAL AREA CA1. M.A. Bowe and J.V. Nadler. Depts. Pharmacology and Neurobiology, Duke Univ. Med. Ctr., Durham, NC 27710.

Several reports suggest that the regulation of NMDA receptor-channel function changes during development. To follow these changes in CA1 pyramidal cells of the rat hippocampus, we have utilized a grease-gap preparation that allows a quantitative pharmacological characterization of responses to excitatory amino acids and their antagonists.

Longitudinal hippocampal slices that included only area CA1 and the retrohippocampal area were prepared from 10-36 day old rats. Each slice was transferred to a two-compartment superfusion chamber and a grease barrier was placed across the CA1-subiculum border. Agonists were applied to the CA1 portion of the slice and the depolarizing responses of pyramidal cells were recorded relative to their axons in the subiculum.

In the absence of Mg^{2+}, the mean EC_{50} for NMDA at 10-13 days of age was 6.8 uM and for AMPA it was 5.6 uM. Both values dropped about 25% between postnatal days 13 and 18. This change may be related to growth of the pyramidal cell dendritic tree. Between postnatal days 10 and 26, 1 mM Mg^{2+} reduced the response to NMDA less than in slices from adult rats. The dose-ratio was only about 2.8, compared with 3.8 in the adult. The effect of Mg^{2+} had reached the full adult level in about half the slices from 30-36 day old rats. These results suggest that NMDA receptor-channel function in CA1 pyramidal cells is less sensitive to Mg^{2+} during development than in adulthood. This may facilitate the NMDA receptor activation needed for learning and brain differentiation. (Supported by NIH grant NS 16064.)

442.7

DIFFERENTIAL EXPRESSION OF CATECHOLAMINERGIC AND ANGIOTENSIN II RECEPTORS IN ASTROCYTIC GLIAL CULTURES FROM NEONATAL AND 21-DAY-OLD RAT BRAINS.

M.K. Raizada,* L. Horky,* and S.P. Baker. Departments of Physiology and Pharmacology & Therapeutics, University of Florida, College of Medicine, Gainesville, Florida. 32610

Catecholaminergic and peptidergic receptors have been implicated to play a role in the development and differentiation of the CNS. However, attempts to investigate the mechanism of their involvement has been hampered, in part, due to the presence of hetogenous cell populations in the CNS. We have established astrocytic glial cells (AGC) in primary culture from the brains of neonatal and 21-day-old rats in order to address this issue. Radioreceptor assays were conducted in AGC from both age groups to quantitate α_1, β-adrenergic, muscarinic and angiotensin II (Ang-II) receptors with the use of [^{125}I]-HEAT, [^{125}I]-iodocyanopindolol (CYP), [^{3}H]-quinuclidinyl benzilate (QNB) and [^{125}I]-Ang II respectively. Comparison of Bmax and Kd's for these receptors indicated significant differences between the neonatal and 21-day-old AGC. Twenty-one day-old AGC expressed a 3.8 fold increase in the Bmax of [^{125}I]-HEAT, a two-fold increase in the Bmax of [^{125}I]-Ang II, and a 45% decrease in the Bmax of [^{125}I]-CYP binding compared with the AGC from neonatal rat brain. In contrast, no change in the Bmax or Kd of [^{3}H]-QNB binding was observed between the AGC of neonatal and 21-day-old brains. These observations indicate that AGC from immature and mature brains express differential receptor levels and may serve as a useful model for the study of CNS development. (Supported by NIH / HL-33610).

442.8

REGULATION OF OPIATE RECEPTORS IN NEONATAL RAT BRAIN FOLLOWING EXOGENOUS DRUG ADMINISTRATION. A. Tempel. Dept. of Neuroscience, Albert Einstein College of Med., Bronx, New York 10461.

Opioid analgesics are well known to produce tolerance and dependence in vivo and desensitization in vitro, although the mechanisms for these phenomenon are not clear. We have found that chronic pre- and postnatal morphine treatment of rat pups produces a significant decrease in brain μ opioid receptor density with no change in receptor affinity. This downregulation is accompanied by tolerance to the actions of morphine as measured by the forepaw withdrawal test (Tempel et al., Dev. Brain Res., 1988). In order to gain insight into the cellular and molecular mechanisms underlying downregulation, the effect of chronic morphine treatment in rat pups on the expression of the principal brain opioid peptide gene, preproenkephalin (PPE) was examined. For chronic prenatal treatment, pregnant dams were implanted with one morphine pellet (75 mg each) on gestational day 15. Pups were sacrificed on postnatal day zero, 4, 7, 14 or 21. For postnatal drug administration, rat pups were injected subcutaneously with either morphine (5 mg/kg daily) or saline for 7, 14, 21 or 28 days. Pair feeding and surrogate fostering techniques were followed. Pups were tested for morphine-induced analgesia or were sacrificed by decapitation following the indicated time. Brains were used for biochemical and molecular analyses. Changes in levels of PPE mRNA were determined by Northern blot analysis and were correlated with regions showing decreases in opiate receptor binding (using receptor autoradiography). These agonist-induced changes may contribute to the symptoms of opiate addiction and tolerance. (Supported by NIH grant DA05440).

442.9

ONTOGENY OF BRAIN GLUCOSE METABOLIC RESPONSE TO COCAINE: A QUALITATIVE AUTORADIOGRAPHIC STUDY. D.L. Dow-Edwards and L.A. Freed Lab of Cerebral Metabolism, SUNY-Health Science Center, Brooklyn, NY, 11203.

We have published long term metabolic changes in rats exposed to cocaine during the preweanling period (Dow-Edwards et al., 1988). However, the acute effects of cocaine on brain glucose metabolism during ontogeny have not been demonstrated.

At either 7, 14 or 21 days of age, nontreated pups were injected with either saline or 25 mg/kg cocaine-HCl, sc and held in an incubator at 35°C. Ten minutes later, [^{14}C] 2-deoxyglucose was injected and the animals were decapitated 45 minutes later. Brains were removed, frozen and processed for autoradiography. Optical density patterns of cortical and subcortical structures were determined using a computerized image analysis system.

At 7 days of age, none of the brain regions appeared to be stimulated, while the thalamus appeared to be metabolically depressed. At 14 days of age, patterns of increased activity appeared in several cortical regions as well as the zona incerta and subthalamic nucleus. At 21 days of age, brain glucose metabolic response to cocaine was similar to that seen in the adult rat (Porrino et al., 1988). The results are discussed in relation to the ontogeny of the behavioral responses to cocaine (Spear & Brick, 1979). Supported by ADAMHA Grant DA04118.

442.10

DEVELOPMENT OF SEX DIFFERENCES IN CATECHOLAMINE METABOLISM IN RAT SYMPATHETIC NEURONS. P. Beaston-Wimmer* and A.J. Smolen (SPON: L. L. Ross). Dept. Anatomy, The Medical College of Pennsylvania, Philadelphia, PA 19129.

At birth, superior cervical ganglia (SCG) of male and female rats contain equal numbers of neurons. No sex difference exists in the content of norepinephrine (NE) in these neurons, in the enzyme activity of tyrosine hydroxylase (TH), or in the expression of TH-mRNA, measured by quantitative in situ hybridization.

During the first two postnatal weeks, natural neuron death in the SCG results in the loss of significantly fewer neurons in males. At this time, neurons from both sexes express equivalent amounts of TH-mRNA, but both TH enzyme activity and NE content per neuron are lower in males. Since the adult sex difference in body weight and sympathetic target mass has not yet been established, the same target mass is innervated by more neurons in males.

In the adult, overall body weight, as well as target organ mass, is significantly greater in males. At this time, the the sex difference in neuron number is maintained, and expression of TH-mRNA remains equivalent in the two sexes. Both TH enzyme activity and NE content per neuron are now equal in males and females.

We conclude that normal amounts of message for TH are expressed even in neurons that are destined to undergo natural neuron death. Further, the development of both TH enzyme activity and NE content in sympathetic neurons appears to depend on target availability, while expression of TH-mRNA appears to develop independently.

Supported by NIH Grants NS15952 and NS21822

442.11

ANDROGEN RECEPTORS ARE DIFFERENTIALLY DISTRIBUTED BETWEEN RIGHT AND LEFT CEREBRAL HEMISPHERES OF THE FETAL MALE RHESUS MONKEY. S.A. Sholl and K.L. Kim*. Wisconsin Regional Primate Research Center, University of Wisconsin, Madison, WI 53715.

In humans there are apparent sex differences in cognitive, spatial and mathematical abilities which may arise as the result of differences in the prenatal development of the cerebral cortex. With this in mind, we have examined androgen receptor (AR), aromatase (AROM) and 5α-reductase (5αR) levels in the brain of Day-70 fetal rhesus monkeys (*Macaca mulatta*). We have reported details of these assays previously (*Endocrinology* 119:1625, 1986; 124:627, 1989.). Receptor and enzyme levels were evaluated in the medial basal hypothalamus (MBH) and in both right (Rt) and left (Lft) temporal (TMP) and frontal (FR) lobes of the cerebral cortex. Five male and 5-6 female fetuses were used in this study. AR levels in FR-Rt of male subjects were higher than levels in FR-Lft (for each and every subject, $P < 0.05$), while in females, there was no consistent pattern in the distribution of the receptor between the two sides of FR. In contrast, AR values in TMP-Lft of male subjects were consistently higher than in TMP-Rt ($P < 0.05$). As with the FR, females exhibited no consistent pattern in the distribution of AR between the two TMP sides. AROM and 5αR levels were similar, regardless of sex, between both sides of the two cortical lobes. In both sexes, the concentrations of AR in the MBH were near the levels observed in the various cortical areas, while AROM and 5αR activities were 3 fold higher in the MBH than in the cortex. The differential cortical distribution of AR in fetal males versus females lends support to the hypothesis that prenatal androgens from the fetal testes may effect the differentiation of sexually dimorphic, side-specific activities in cortical activity. In addition, we recently demonstrated high 5αR activity in the corpus callosum (*Endocrinology* 124:627,1989) which may serve to convert testosterone to its more active metabolite, DHT, and concentrate DHT in the cerebrum. (Supported by NIH Grant HD-18865.)

ALZHEIMER'S DISEASE II

443.1

METABOLITES ELEVATED IN ALZHEIMER'S BRAIN ALTER MEMBRANE PROPERTIES. **J.W. Pettegrew, D. McKeag* and S. Strychor*. Laboratory of Neurophysics, Departments of Psychiatry and Neurology, University of Pittsburgh School of Medicine, Pittsburgh, PA 15261**

In vitro and in vivo ^{31}P NMR studies demonstrate that phosphomonoesters (PME) such as phosphocholine (PC), phosphoethanolamine (PE) and L-phosphoserine (PS) are elevated (5-7 mM) in Alzheimer's disease brain. The present study examines whether PME alter plasma membrane molecular dynamics. Erythrocytes were obtained from 11 normal adult volunteers and labelled with fluorophores that monitor molecular motion in specific areas of the membrane. The erythrocytes were incubated for 30 minutes at 37°C with either phosphate buffered saline or a given concentration (100 μM, 500 μM, 1.0 mM) of one of the PME (PC, PE or PS) or a combination of PME. Following the incubation period, anisotropy values were determined. Compared to buffer the PME increased molecular motion on the surface ($p = 0.0001$) and in the hydrocarbon core ($p = 0.01$) and decreased molecular motion at the aqueous-hydrocarbon interface ($p = 0.0001$). The PME could therefore alter membrane molecular dynamics in CNS cells and alter the function of membrane proteins such as receptors, channels and second messenger cascades. The circulating erythrocyte also could be a useful "reporter cell" for CNS molecular pathology and monitor disease progression and the response to therapeutic interventions.

443.2

CHROMOGRANIN A-LIKE IMMUNOREACTIVE NEURONS ARE RESISTANT TO TANGLE FORMATION AND DEATH IN ALZHEIMER'S DISEASE. D.G. Munoz and C. Caro*. Dept. of Pathology, University of Saskatchewan, Saskatoon, Sask., S7N 0W0

Chromogranin A-like immunoreactivity (CgA-li), as defined by the monoclonal antibody LK2H10, is widespread, but restricted to certain neuronal populations in the human brain. The distribution of CgA-li neurons in the hippocampus coincides with the pattern of resistance to seizure-induced damage (Munoz et al ASN 1988;14: 983). We have studied the entorhinal cortex and hippocampus of 5 patients with Alzheimer's disease (AD) and 12 controls. The principal neurons of entorhinal layers II and IV lacked CgA-li; layer III showed weak and layers V-VI moderate CgA-li. In addition, sparse, intensely CgA-li neurons were scattered in layers III to VI. Neurofibrillary tangles in AD predominantly occurred in layers (II and IV) lacking CgA-li neurons. Intense and moderately CgA-li neurons showed significantly better survival in AD than CgA-li negative neurons. Similarly, in the hippocampus, CgA-li neurons in CA2 survive and rarely develop tangles, whereas CgA-li negative CA1 and subicular neurons develop tangles and die. Thus, although CgA-li neurons contribute neurites to almost all plaques (Munoz, Neurology 1989;39: S396), their somata are relatively resistant to tangle formation and death. These data support a role for excitotoxicity in the pathogenesis of AD.

443.3

THE DISTRIBUTION OF CHOLECYSTOKININ-LIKE IMMUNOREACTIVITY (CCKLI) IN ISOCORTEX IN NORMALS AND ALZHEIMER'S DISEASE (AD). D.G. Cole* and N.W. Kowall (SPON: S.K. Kostyk). Neurology Service, Massachusetts General Hospital, Boston MA 02114.

We studied the pattern of CCKLI in the isocortex of brains of aged controls and patients with AD using immunoperoxidase methods and well-characterized polyclonal CCK antiserum (INCstar). In normals we found a characteristic laminar distribution of CCKLI terminals, with strong staining of layers II, IV, and VI, and trilaminar staining in layer I. The major regional variation in this theme was in area 17, where 4A stained mildly, 4B weakly, 4Cα strongly, and 4Cß weakly, with diminution of clear distinction between layers V and VI. Cell staining was rare in the perirolandic and striate regions but common in inferior temporal and medial infero-frontal areas. Fiber staining was rare. In AD brains we found the trilaminate layer I staining lost while the remainder of the laminar pattern was preserved. Layer IV often stained less prominently than in normals. Senile plaques predominated in layers II and III, and they were more plentiful in higher association cortex than primary sensory and motor cortex. Occasional dystrophic fibers were seen. Cell staining was unchanged compared to normals. Our results establish the distinctive pattern of CCKLI in homotypical and heterotypical isocortex and the changes in this pattern seen in AD. The absence of a simple proportional correlation between the distribution of senile plaques and terminals suggests preferential involvement of a subset of CCK neurons in AD.

443.4

INHIBITION OF HISTAMINE METABOLISM BY THA (9-AMINO-1,2,3,4-TETRAHYDROACRIDINE). P. Cumming, P.B. Reiner and S.R. Vincent. Kinsmen Laboratory of Neurological Research, Department of Psychiatry, The University of British Columbia, Vancouver, B.C., V6T 1W5, Canada.

Chronic administration of 9-amino-1,2,3,4-tetrahydroacridine (THA) has been reported to produce clinical improvement in some cases of Alzheimer's disease. The anticholinesterase activity of THA provided the original rationale for using this drug. However, the pathology of Alzheimer's disease is not restricted to cholinergic neurons. Decreased cortical histamine levels and neurodegenerative changes in the histaminergic tuberomammillary nucleus have been reported in this disease. We noted the structural similarity of THA to quinacrine and related antimalarial drugs which are potent inhibitors of histamine-N-methyl transferase (HNMT), the enzyme responsible for histamine catabolism in the brain. We therefore examined the effects of THA on partially purified rat brain HNMT assayed in the presence of 10 μM tritiated S-adenosyl methionine at histamine concentrations from 1 to 10 μM. Under these conditions, the enzyme exhibited a K_m for histamine of 3.5 μM. THA was found to be one of the most potent competitive inhibitors of HNMT yet described, with a K_i of 36 nM. In contrast, the structurally related compound 4-aminopyridine was 1000-fold less potent. Since therapeutic effects of THA have been reported with serum levels as low as 30 nM, substantial inhibition of histamine metabolism would be expected in the clinical situation. Thus, some of the clinical efficacy of this drug may be related to its ability to augment histamine neurotransmission in the brain.

443.5

LOSS OF GUANINE NUCLEOTIDE-SENSITIVE HIGH AFFINITY AGONIST BINDING TO M1 MUSCARINIC RECEPTORS IN ALZHEIMER'S DISEASE. D.D. Flynn and D.C. Mash, Departments of Pharmacology and Neurology, University of Miami School of Medicine, Miami, FL 33101.

Diminished cholinergic neurotransmission is thought to play a major role in the development of Alzheimer's disease psychopathology. The failure of cholinergic replacement therapies to ameliorate specific features of the symptomatology may reflect a diminished responsiveness of postsynaptic muscarinic receptors (putative M1 receptor subtype) to direct and indirect-acting cholinergic agnoists, since the number of these receptor sites appears to be unchanged throughout the course of the disease. A selective reduction in M1 receptor-G protein coupling has been suggested in a preliminary study which used one point carbachol-[^{3}H]-pirenzepine assays on cerebral cortex homogenates from AD subjects (Perry, E.K., et al., Neurosci. Lett. 82:277, 1987). Using full carbachol-[^{3}H]-pirenzepine displacement assays, we demonstrate that M1 receptors show nearly a 100-fold difference in their high (K_H) and low (K_L) affinity agonist states. High affinity binding is converted to low affinity by the addition of the nonhydrolyzable GTP analog, GppNHp (0.2 mM). Our results further demonstrate that the proportion of M1 receptors in the high affinity agonist state is significantly diminished in AD, with a concomitant loss in sensitivity of the K_H state of the receptor to GppNHp. We are currently attempting to correlate variations in agonist affinity states of the M1 receptor subtype in AD with age of onset, disease duration and severity. Supported by NS 19065 and NS 25785.

443.6

A COMPARISON OF THE DISTRIBUTION OF CEREBRAL GLUCOSE METABOLISM AND OF MUSCARINIC RECEPTORS IN VIVO IN DEMENTIA. D.R. Weinberger, U. Mann*, R.C. Reba*, D.W. Jones*, R. Coppola, R.E. Gibson* and T.N. Chase. CBDB, NIMH Neurosciences Center at St. Elizabeths, Washington, D.C. 20032.

PET studies of glucose metabolism in patients with Alzheimer's Disease (AD) and in patients with Pick's Disease (PK) often reveal reduced metabolism in parietal and frontal cortices, respectively. We have recently observed with SPECT similar regional patterns of uptake of the muscarinic receptor antagonist ^{123}IodoQNB. To evaluate the comparability of the data from these techniques, 10 subjects (4 AD, 2 PK, 4 controls) underwent both an ^{18}FDG PET scan and a high resolution SPECT scan. SPECT was performed approximately 20 hours after injection of ^{123}IodoQNB when nonspecific binding appears to be negligible. Overall, similar patterns of focal defects were seen. On the glucose PET scans, reduced metabolism was observed in parietal and frontal cortex. With SPECT, the distribution of muscarinic receptor binding was reduced in parietal cortex in AD patients and in frontal cortex in PK patients. However, one of the PK patients had basal ganglia hypometabolism despite good ligand uptake and the other had a greater anterior-posterior gradiant in ligand uptake than in metabolism. Asymmetries in posterior cortical regions tended to be more striking on the QNB images. ROI analysis on 4 patients supported these observations. These preliminary data suggest that ^{18}FDG PET and ^{123}IodoQNB SPECT provide different information about brain function in dementia and that the muscarinic receptor binding results reflect more than partial volume artifacts or flow dependent ligand uptake.

443.7

ANTIBODIES IN THE CEREBRO-SPINAL FLUID (CSF) OF ALZHEIMER'S DISEASE PATIENTS: INVESTIGATION USING CHOLINERGIC NEURONAL CULTURES A.Dahlström, A.Wigander*, K.Lundmark* and A.McRae, Institute of Neurobiology Univ. of Goteborg Goteborg, Sweden 40033

Our previous findings have indicated that there is an antibody in the CSF from some AD patients which recognizes cholinergic neuronal populations in the rat central nervous system (CNS). In order to further investigate the specificity of this CSF antibody neuronal cultures of cholinergic cells were employed. Cholinergic cultures were prepared from dissociated cells from the medial septum region of 17 day old fetal rats and were maintained in culture for 3 weeks. The cultures were fixed with 4% paraformaldehyde and then were doubled stained with AD CSF and anti nerve growth receptor antiserum (a-NGF-R). A considerable number of cells were doubled labeled with AD CSF and a-NGF-R antiserum. This supports the concept that an antibody in the CSF from AD patients recognizes cholinergic neurons in culture as well as in the rat CNS. Addition of a-NGF-R (1/200) to cholinergic cells destroyed the neuronal population within 5 days. Addition of CSF samples from AD, and other demented patients did not destroy cholinergic cells. However when a-NGF-R antiserum was added to neurons incubated with patient CSF samples only those cultures incubated with AD CSF survived the destruction induced by a-NGF-R antiserum. Our results support the concept that there is an antibody in CSF from AD patients which recognizes cholinergic neurons and that this antibody counteracts the destructive effects of a-NGF-R antiserum to cholinergic neurons. These data provide further support to our hypothesis that the presence of IgG in the CSF of AD patients may participate in the pathogenesis of this neurodegenerative disease.

443.8

ANTICHOLINERGICS REINSTATE SENSORIMOTOR DEFICITS IN RATS WITH NEOCORTICAL DAMAGE: PROTECTIVE EFFECTS OF SABELUZOLE. M. De Ryck, H. Duytschaever*, G. H. C. Clincke*, A. Wauquier* and P. A. J. Janssen*, Janssen Research Foundation, Department of Neuropsychopharmacology, B-2340 Beerse, Belgium.

Unilateral photochemical infarction of the sensorimotor cortex in rats produced enduring limb placing deficits. When such rats (n=120) traversed narrow beams, the contralateral, but not ipsilateral, hindlimb frequently slipped off. With consecutive test sessions, performance improved to an asymptotic, residual deficit, which ranged from severe (e.g., 30-40 contralat. versus 0-2 ipsilat. errors) to slight (e.g., 2-4 errors). Atropine SO_4, at a dose that did not affect performance of normals (10 mg/kg ip), increased contralateral placing errors in infarcted rats from 10 (5-34) to 33 (23-66) [median (interquart. range); n=16; Wilcoxon test, $p < 0.001$]. Scopolamine HBr (1 mg/kg ip) yielded similar effects, which are central in origin, as the quaternary salts of atropine and scopolamine did not alter performance. None of these treatments produced gross sensorimotor dysfunctions. Sabeluzole (10 mg/kg po), a cognitive enhancer in several species, including man (Clincke, G.H.C., et al., *Psychopharmacology*, 94:52, 1988), diminished the deleterious effects of atropine in brain-damaged rats to 17 (9-44) errors ($p < 0.01$). Sabeluzole was significantly active within the dose range of 5-20 mg/kg po. Cholinergic forebrain mechanisms may be more vulnerable to cholinergic blockade after cortical damage. The protective effects of sabeluzole on such mechanisms, and their relationship to age- or Alzheimer's disease-related brain disorders, need further clarification.

443.9

SPECIFIC ALTERATIONS IN CALCIUM BINDING PROTEIN GENE EXPRESSION IN NEURODEGENERATIVE DISEASES. A. M. Iacopino* and S. S. Christakos, Biochemistry and Molecular Biology, UMDNJ-New Jersey Medical School, Newark, NJ 07103.

We have utilized a specific cDNA for the vitamin-D-dependent calcium binding protein, calbindin-D_{28k}, in order to examine changes in its gene expression in the aging rat and human brain as well as in 3 neurodegenerative diseases (Parkinson's, Huntington's, Alzheimer's). Changes in calbindin-D_{28k} gene expression were characterized by Northern and slot blot analysis (all blots were reprobed with B-actin and calmodulin cDNAs to determine specificity). Using gross brain regions in the aging rat, significant decreases in calbindin-D_{28k} mRNA were found in the cerebellum (4-fold) and subcortical area (9-fold) but not in the cerebral cortex. Using more specific brain areas in the aging human brain, significant decreases in calbindin mRNA levels were seen in the cerebellum (6-fold), corpus striatum (5-fold), and nucleus basalis (7-fold) but not in the neocortex, hippocampus, locus coerulus, amygdala, or nucleus raphe dorsalis. Comparison of diseased human brain tissue with age/sex matched controls yielded significant, specific decreases in calbindin-D_{28k} mRNA for substancia nigra (Parkinson's, 5-fold), corpus striatum (Huntington's, 8-fold), and nucleus basalis (Alzheimer's, 8-fold) but not in the amygdala, neocortex, or locus coerulus. Since the calbindin-containing neurons in these areas are particularly affected in each of the disease processes, it is suggested that a decrease in calbindin may lead to a failure of intraneuronal calcium homeostasis and calcium-mediated irreversible cytotoxic events during the pathological processes.

443.10

ALTERED cAMP-DEPENDENT PROTEIN PHOSPHORYLATIONS IN POSTMORTEM BRAIN TISSUES AFFLICTED BY ALZHEIMER'S DISEASE. W. Wallace, K.M. Parks*, L. Bierer*, D. Perl*, and V. Haroutunian* Department of Psychiatry and Fishberg Center For Neurobiology, Mount Sinai School of Medicine, New York, N.Y. 10029

We have examined protein phosphorylation patterns in human postmortem tissues by the back phosphorylation assay, in which endogenous protein kinase activities are measured as the in vitro incorporation of phosphate into various endogenous phosphoproteins. The cAMP dependent phosphorylations were measured as the amount of phosphate incorporated into two specific and well characterized substrates, Synapsin I and pyruvate dehydrogenase (PDH). Although the degree of phosphorylation varied with the tissue sample, this variability was inherent to the sample and not due to the assay, itself. The sample variability did not correlate with either age of the brain ($r=-.17$ $p<.61$ $n=11$ range =66 to 97 years) or postmortem interval suffered by the tissue ($r=-.23$, $p<0.37$, $r=17$ range =84 to 910 minutes). Homogenates from the temporal cortex (TC) of Alzheimer's disease and age-matched control brains exhibited a 2.6-fold difference in phosphorylation of Synapsin I (267 vs 104 fmols phosphate/mg total homogenate protein, $n=7$, $p<.03$). No differences were observed in the phosphorylation of PDH in the same tissues (144 vs 120 fmols phosphate/mg total protein). In addition, no differences were detected with either substrate within the cerebella of the two groups. Numerous other proteins exhibited similar differences inphosphate content as judged by autoradiography. These differences are not due to the absolute amount or structural integrity of the substrates. Whether these differences in phosphorylation of Synapsin I in the TC are due to altered endogenous cAMP kinase activity or altered in situ state of phosphorylation of the substrate proteins is currently being determined. Funded in part by ADRDA.

443.11

TOPOGRAPHY OF GLUCOSE METABOLIC DEFICITS IN ALZHEIMER'S DISEASE: EVIDENCE FROM CROSS-SECTIONAL AND LONGITUDINAL STUDIES. G. Smith., M. deLeon, A. George, A. Kluger, S.H. Ferris, B. Reisberg, N. Volkow, A. Wolf. Depts. of Psychiatry and Radiology, New York Univ. Med. Ctr. 10016 and Dept. of Chemistry, Brookhaven National Labs.

PET VI and ^{11}C 2-DG was used to describe the location and progression of the glucose metabolic deficits in Alzheimer's disease (AD). 34 AD patients who met NINCDS-ADRDA criteria and 21 age-matched controls were evaluated. Patients were stratified into mild (n=14) and moderate-severe (n=20) cognitive impairment groups. 17 of the subjects were examined at 2 to 3 years follow-up.

In comparing the mild AD group to the controls, statistically significant differences were observed (15-19%) in parietal, temporal, somatosensory, frontal, and occipital areas. The moderate-severe cases showed even greater widespread change, with further reductions (an additional 8-12%) in the areas involved in mild AD. Deficits in basal ganglia and thalamus was observed in the moderate-severe group, relative to controls. The longitudinal data confirm that the regions which show progressive deterioration across severity levels also declined significantly within patients.

The presentation of diffuse cerebral metabolic deficits, accentuated in temporal, parietal and frontal cortices is more consistent with the distribution of neurotransmitter deficits and neuropathological markers in AD. Supported by RO1 MH43965

443.12

GANGLION CELL LOSS IN MACULA OF PATIENTS WITH ALZHEIMER'S DISEASE Y. Torigoe, J.C.Blanks, R.H.I.Blanks, J. Cabaret*, Department of Anatomy and Neurobiol., Univ. Calif. Irvine, Irvine, CA 92717 and Estelle Doheny Eye Inst., Dept. Ophthalmol., Univ. Southern Calif., Los Angeles, CA 90033.

Ten retinas from 8 patients with Alzheimer's disease (AD) were evaluated to determine the size and distribution of ganglion cells in the macula. Data from patients ranging in age from 79 to 89 yr (mean=83) were compared to 7 age-matched controls (range, 60-85 yr; mean=73). Eyes were removed 1.5-6 hrs after death and fixed in 2% paraformaldehyde and 2% glutaraldehyde. The diagnosis of AD was confirmed by the presence of plaques and tangles in representative brain regions. All neurons within the ganglion cell layer (GCL) were drawn, digitized and split-cell fragments corrected to obtain accurate cell numbers and diameters. Retinal ganglion cells (RGCs) in the control, aged retina are medium-sized; their frequency distribution histogram is unimodal, symmetrical and comparable across the population of AD patients (13.2±1.4 um) and controls (14.2±1.7 um). The fovea (0-6° eccentricity) of AD patients shows a significant loss of neurons within the GCL and there is a tendency towards smaller numbers in the parafoveal area (eccentricity 6-9°). The plexiform layers, inner and outer nuclear layer, and photoreceptor cell layer appear normal in thickness and cell number. GCL neuronal density (neurons/mm^2) for AD patients is 4,007±1,839 and 8,591±970 for eccentricities of 0-3° and 3-6°, respectively, which is significantly lower (two sample t-test) than counts of 9,855±3,446 and 12,896 for controls. The difference at eccentricities of 6-9° between AD patients (14,491±7,443) and controls (15,229±4,272) is not significant. Thus, AD patients have lost 59% (range 35-82%) of the neurons of the GCL from 0-3° of the foveola and 33% (range 25-45%) from 3-6°. These results confirm earlier findings of retinal defects in AD patients, and demonstrate a significant loss of RGCs from the macula. Neuronal cell counts from retinal whole-mounts of these patients are underway to assess possible degeneration in the peripheral retina. The loss of RGCs (and possibly displaced amacrine cells located in the GCL) from the macula provides the anatomical substrate for the documented visual deficits found in AD.

TRAUMA III

444.1

BENEFICIAL EFFECTS OF PLATELET ACTIVATING FACTOR (PAF) ANTAGONIST IN EXPERIMENTAL NEUROINJURY IN RATS. K.U. Frerichs,* P.J. Lindsberg,* J.M. Hallenbeck* and G.Z. Feuerstein. (SPON:B.Cox) Dept. of Neurology, USUHS, Bethesda, MD 20814

The pathomechanisms of secondary brain damage after ischemic or traumatic brain injury are far from understood. Since PAF has been recently discussed to be a potential mediator in neuroinjury, we studied the effects of the selective PAF antagonist BN50739 on lesion volume, local CBF (lCBF)(laser doppler flowmetry) and blood brain barrier integrity (evans blue albumin (EBA)) in a model of focal and highly discrete cortical neuroinjury (thermal; Nd:YAG laser) in anesthetized rats. Expansion of the lesion volume within the first 24h after injury in vehicle injected controls was reduced by pretreatment with BN50739 (10mg/kg) by 25% (n=10,p<0.01) at 24h. The progressive hypoperfusion (lCBF: 40±8% of baseline,n=7) 1mm from the lesion within the first two hours after insult was partially prevented by BN50739 (lCBF: 80±7% of baseline,n=5,p<0.05) at 2h. Improvement of lCBF by BN50739 was associated with decreased extravasation of EBA (p<0.05). The present study provides supporting evidence, that PAF may be a key mediator in the evolution of hypoperfusion dependent brain injury; PAF antagonists therefore may provide significant therapeutic protection in arresting secondary brain damage following cerebral ischemia and neurotrauma.

444.2

ROLE OF PLATELET-ACTIVATING FACTOR (PAF) IN THE PATHOPHYSIOLOGY OF TRAUMATIC BRAIN INJURY. A.I. Faden, P. Tzendzalian*, M. Lemke and F. Valone*. Depts. of Neurology and Medicine, Univ. of CA, San Francisco, and VA Medical Center, San Francisco, CA, 94121.

PAF, or 1-O-alkyl-2(R)-acetyl-sn-glycero-3-phosphocholine, has been implicated in the pathophysiology of cerebral ischemia. BN 52021 is a PAF antagonist, isolated from Ginko biloba extract, which reduces pathophysiological effects of PAF and limits biochemical and behavioral effects in brain ischemia models. In the present studies, we examined whether fluid-percussion-induced traumatic brain injury (TBI) causes release of PAF and whether pretreatment with BN 52021 can modify outcome. Pentobarbital-anesthetized rats were subjected to standardized trauma (2.85 atm). In one experiment, brain tissue from the trauma region was removed 5 min after injury. PAF levels were quantified by rabbit platelet aggregation; specific PAF antagonists and HPLC purification were used to confirm that the bioactive material was PAF. After TBI, PAF content was increased (7.9 ± 1.7 vs. 4.7 ± 2.4 fmoles/sample, each n=4). In other experiments, animals were randomly (n=21) assigned to treatment with BN 52021 (1 mg/kg or 10 mg/kg) or equal volume vehicle, given i.v. 15 min pretrauma and 2 h posttrauma. Animals treated with BN 52021 showed a significant, dose-related improvement in behavioral recovery at 2 weeks posttrauma. Taken together, these data suggest a potential role for PAF in the pathophysiology of TBI.

444.3

DYNORPHIN A ATTENUATES NMDA RECEPTOR-MEDIATED CORTICAL NEURONAL INJURY IN VITRO. [1]D.W. Choi, K. Rose*, J.H. Weiss and [2]A.I. Faden. [1]Dept. of Neurology, Stanford Univ. Med. Sch., Stanford CA 94305 and [2]Dept. of Neurology, Univ. California, San Francisco CA 94121.

Dynorphin A 1-17 (Dyn) is potently neurotoxic in vivo and may play a role in the pathogenesis of traumatic central nervous system injury. Whether exposure to Dyn injures neurons directly, or indirectly (for example through vasoconstriction), has not been established. We investigated Dyn A for neurotoxic efficacy in murine cortical cell cultures, and found that 24 hr exposure to Dyn alone, at concentrations up to 60-100 μM, produced little neuronal or glial injury.

In fact, addition of 60 μM Dyn to these cultures actually substantially reduced the neuronal degeneration induced by exposure to 20 μM NMDA for 24 hr. In one experiment, cultures exposed to NMDA alone developed by the next day a media lactate dehydrogenase signal of 283 +/- 16.5 U/ml (SEM, n = 4) over wash control; sister cultures exposed to NMDA in the presence of 60 μM Dyn showed a signal of only 27.3 +/- 15.4 U/ml (n = 4) (different at $p < 0.001$). These data support the idea that the neurotoxicity of Dyn in vivo is indirectly mediated, and furthermore suggest that high concentrations of Dyn can actually reduce NMDA receptor-mediated neurotoxicity.

444.4

PRETREATMENT WITH MK-801 REDUCES BEHAVIORAL DEFICITS FOLLOWING TRAUMATIC BRAIN INJURY (TBI) IN RATS. B.G. Lyeth, L.W. Jenkins, R.J. Hamm, L.L. Phillips, C.E. Dixon, J.J. Yao*, H.F.Young*, J.F. Stubbins*, G.L. Clifton* and R.L. Hayes. Division of Neurosurgery, Medical College of Virginia/Virginia Commonwealth University, Richmond, VA 23298

Several lines of evidence suggest that excitatory agonist-receptor interactions at muscarinic (Lyeth et al., Brain Research 452:39, 1988) and NMDA (Hayes et al., J. Neurotrauma 5:287, 1988) sites contribute to pathophysiological consequences of TBI. The present study examined the effects of the non-competitive NMDA antagonist, MK-801 on behavioral deficits associated with fluid percussion TBI in the rat.

Rats (n=10/group) were administered either saline or MK-801 (0.1, 0.3 or 1.0 mg/kg, i.p.) 15 min, and Metofane 5 min prior to moderate fluid percussion TBI. Rats were ventilated as necessary following injury. Rectal temperature was monitored prior to and for 40 minutes after injury. Brain temperature was monitored on 5 additional uninjured rats treated with 0.3 mg/kg MK-801. Behavioral assessments were made for 5 days after injury. Treatment with 0.3 mg/kg MK-801 resulted in significantly less weight loss and significantly reduced beam-balance and beam-walking deficits. MK-801 did not significantly alter rectal or brain temperature. These data suggest that NMDA agonist-receptor interactions contribute to the pathophysiology of brain injury. Supported by NS 21458, NS 12587 and Merck Sharp & Dohme.

444.5

MK801 NEUROPROTECTIVE EFFECTS IN A RODENT MODEL OF PEPTIDE INDUCED SPINAL CORD INJURY: A HISTOLOGICAL STUDY D.D. Rigamonti, A. Martinez-Arizala, R.F. Genovese, J.W. Holaday, and J.B. Long. Dept Med Neurosci, Walter Reed Army Inst Res, Wash, DC 20307.

Dynorphin A 1-17 (DYN) causes neuropathological changes associated with hindlimb paralysis, nociceptive loss and spinal cord ischemia when injected into the lumbar cistern. Since NMDA receptors have been implicated in the pathophysiology of ischemic CNS injury, we examined the effects of MK801, a non-competitive NMDA receptor antagonist, on DYN-induced histopathological changes. Adult S-D rats were divided and injected intrathecally as: Group I-saline, Group II-DYN, and Group III-DYN+MK801. After neurological evaluation for 72 hours, the rats were perfused, the lumbosacral cord was removed, and serial cross-sections stained using H & E, Nissl, Kluver-Barrera and Bielschowsky techniques. Samples were evaluated by three blinded observers. Interrater agreement and differences between groups were statistically analyzed. Spinal tissue from I appeared normal while tissue from II exhibited lumbosacral cord necrosis. The injury was characterized by widespread ischemic cell changes, neuronal cell loss and axonal degeneration in the gray and adjacent white matter, with diffuse gliosis and cellular infiltration. Tissue from III exhibited small islands of necrotic tissue or slight ischemic changes, restricted to the gray matter. In addition, there was mild gliosis and edema. Thus, MK801 improved neurological and neuropathological outcome in DYN-treated animals.

444.6

MUSCARINIC AND NMDA RECEPTOR BLOCKADE REDUCES POST-ISCHEMIC EEG SPIKE FREQUENCY FOLLOWING TBI AND ACUTE SECONDARY ISCHEMIA. L. Jenkins, B. Lyeth, D. DeWitt, R. Hamm, L. Phillips, H. Young*, G. Clifton*, and R. Hayes. Division of Neurosurgery, Medical College of Virginia, Richmond, VA 23298.

Following mild traumatic injury (TBI) subsequent brain vulnerability to cerebral ischemia is enhanced (Brain Research 477:211,1989) and receptor related since combined muscarinic and NMDA receptor blockade can significantly reduce enhanced delayed neuronal death after TBI followed by acute or delayed cerebral ischemia (J Neurotrauma 5:303,1988). We examined the effect of muscarinic and NMDA receptor blockade on the frequency of post-ischemic EEG spike activity as one possible mechanism for neural protection. Wistar rats (250-300g) were subjected to mild TBI followed 1 hr later by 6 min of forebrain ischemia. Two groups (N=6/group) were examined, one without drug treatment and another group with simultaneous i.p. 1mg/kg scopolamine and 4mg/kg phencyclidine (PCP) 15 minutes prior to TBI. Drug treated rats (21.1 ± 0.8 spikes/min) demonstrated a 27% reduction in post-ischemic spike frequency compared to the untreated group (29.1 ± 0.9 spikes/min). Regenerative neuronal spike activity and post-ischemic calcium entry (J Cereb Blood Flow & Metabol 8:799, 1988) are known to be influenced by both NMDA receptor linked inward calcium currents and muscarinic receptor linked outward potassium currents (TINS 10:59,1987). Thus, neural protection by combined scopolamine and PCP in mild TBI followed by acute secondary ischemia may be related to receptor modulation of these Ca^{2+} and K^+ channel protein conductance states.

Supported by NS19950 and NS12587.

444.7

EFFECT OF PHENCYCLIDINE (PCP) ON CENTRAL CHOLINERGIC ACTIVITY FOLLOWING TRAUMATIC BRAIN INJURY (TBI). S.E. Robinson, E.K. Enters*, M.G. Posner*, S.D. Foxx*, R.M. Martin*, C.A. Gyenes*, and T.R. Davis*. Dept.of. Pharmacology & Toxicology, Medical College of Virginia, Richmond, VA 23298-0613.

TBI has been found to produce changes in acetylcholine (ACh) turnover at early times following injury (Saija et al., Brain Res. 452: 303, 1988). Because PCP, a noncompetitive NMDA antagonist, reduces some of the behavioral deficits following TBI (Hayes, R.L,et al,Soc. Neurosci. Abstr. 17: 1254, 1987), we examined the effect of treatment with PCP prior to TBI on ACh turnover in the dorsal hippocampus, frontal cortex, and caudate nucleus, areas containing cholinergic and excitatory amino acid innervation. ACh turnover rate (TR_{ACh}) was measured by a mass fragmentographic technique, which quantitated the relative incorporation of deuterium label from infused phosphorylcholine into ACh and choline. Male Sprague-Dawley rats (275-290 g) were surgically prepared under Equithesin anesthesia 24 h prior to injury, and were administered either saline or PCP (4mg/kg) i.p. 15 min and methoxyflurane anesthesia 5 min prior to injury (2.2 atm) or sham-injury. Rats were infused through the tail vein at a constant rate with $^2H_9$-phosphorylcholine (15 μmol/kg/min) beginning 3 min after TBI and euthanized by focussed microwave irradiation (1.2 sec, 9.8 kW) 12 min after TBI. TBI reduced dorsal hippocampal TR_{ACh} to 22% of sham control. Moreover, PCP pretreatment prevented a significant reduction in TR_{ACh} after TBI. TR_{ACh} was not significantly affected in the other 2 areas. These results suggest that activation of NMDA receptors may lead to changes in cholinergic activity following TBI. These changes may contribute to long-term behavioral changes that occur after TBI. (Supported by NS 24413 and NS 07288).

444.8

TRAUMATICALLY INDUCED BLOOD-BRAIN BARRIER DISRUPTION: A CONDUIT FOR THE PASSAGE OF CIRCULATING EXCITATORY NEUROTRANSMITTERS. J.T. Povlishock and B.G. Lyeth Depts. of Anatomy and Surgery, Medical College of Virginia, Virginia Commonwealth University, Richmond, VA 23298

In rodent, traumatic brain injury (TBI) results in the release of excitatory neurotransmitters that participate in pathological agonist receptor interactions (Brain Res. 542:39,1988). It has been assumed that all excitatory neurotransmitters are derived from synaptic terminals, however, it is possible that some reach the brain via a compromised-blood-brain barrier (BBB). To test this, BBB status was assessed in brain regions previously linked with functional change. Rats were subjected to mild TBI and were processed for the immuno cytochemical detection of various circulating immunoglobulins and neurotransmitters normally excluded by the BBB. TBI resulted in the passage of immunoglobulins and excitatory amino acids such as glutamate into the interstices of both the cerebral cortices and dorsal hippocampi. These substances crossed the BBB without any overt endothelial change. Their passage was transient and interestingly was blunted by the administration of superoxide dismutase. Supported by NS-20193

444.9

RAPID GENOMIC RESPONSE TO LOCAL INJURY IN HIPPOCAMPAL DENTATE GRANULE CELLS. A.J. Cole, P.F. Worley, and J.M. Baraban. Dept. of Neuroscience, Johns Hopkins University School of Medicine, Baltimore, MD 21205.

Recent studies suggest that brain injury elicits a rapid increase of c-fos protein in both neurons and glia. In our studies of the regulation of c-fos and other immediate early genes in brain, we noted that sham Hamilton syringe injections frequently elicited an increase in mRNA levels. To study this genomic response to injury in a systematic fashion, we determined that while introduction of fine glass recording microelectrodes had no effect, penetration of the hippocampus with a 28-gauge stimulating electrode reliable caused an increase in c-fos and zif/268 mRNAs. The increase is apparent as early as 5 minutes after injury, but not after 1 minute. Increased mRNA levels in the hippocampus are not produced by limiting the injury to overlying cortex, or to other brain areas such as cerebellum or striatum. By contrast, in a number of experiments, we noted widespread increases in these mRNA levels throughout ipsilateral, but not contralateral, cortex, regardless of the site of injury. These findings indicate that direct intracerebral injections may be sufficient to activate a neuronal genomic response. Moreover, this type of simple penetrating head injury offers a model system to elucidate the mechanisms of both local and hemispheric genomic responses.

444.10

Pressure Induced Trauma in Cell Cultures. E.J. Murphy* and L.A. Horrocks. Department Physiological Chemistry, The Ohio State University, Columbus, OH 43210.

In order to develop a cell culture model for spinal cord injury, mechanical trauma is produced by an increase in atmospheric pressure. A short pressure duration results in reacylation of released fatty acids (FA). The injury becomes sustainable by increasing the pressure duration to 3 min. In the following experiments pressure was varied (duration 3 min) between 5, 10, 15 and 20 atm. Recovery times of 1 and 10 min were used to monitor reacylation. In ROC-1 oligodendroglia cultures the maximum FA increase (2.7 fold over controls) occurs at 5 atm 10 min. At 15 and 20 atm the 1 min levels are greater than the 10 min values indicating reacylation. Arachidonic acid (20:4 n-6) was increased 9 fold over controls at 5 atm 10 min. In human umbilical vein endothelial cells (HUVE) the greatest FA release (2.3 fold over controls) occurs at 15 atm 1 min. FA levels return to normal by 10 min. 20:4 n-6 is increased 13 fold over controls at 5 atm 1 min and returns to normal by 10 min. In both cultures the primary FA released are 16:0, 18:0 and 18:1, and to a lesser extent 18:2 and 18:3 (n-6 and n-3). In ROC-1 cells a sustainable injury was produced, however, in HUVE cells only a transient perturbation of the membrane occurs. Support from NS-10165 and Doerenkamp-Zbinden Foundation.

444.11

THE EFFECT OF A DIRECT CURRENT FIELD ON CHRONICALLY INJURED MAMMALIAN SPINAL CORD AXONS. M.G. Fehlings * C.H. Tator (SPON: E. Theriault) Playfair Neurosci. Unit, Univ. Toronto M5T 2S8

Recent studies indicate that direct current (DC) fields promote the recovery of acutely injured axons; however, the effect of DC fields on chronically injured spinal axons is unknown. In this study, DC stimulators (14 uA; cathode caudal to lesion) were implanted in 30 rats (15 with functioning stimulators; 15 with sham units) 4 weeks after a 53 g clip injury of the T1 cord. Hindlimb function was assessed by the inclined plane method. At 12 weeks after injury, motor and somatosensory evoked potentials were recorded and HRP was introduced into the T6 cord. Labeled cells were counted in the brainstem and motor cortex and axons were counted at the injury site.

The inclined plane scores, evoked potentials, and axon counts of treated and control rats were similar ($p>0.05$). However, counts of HRP-labeled neurons were significantly higher in treated rats in the red nucleus (96.8 ± 29.5 vs 24.6 ± 7.5; $p<0.002$); reticular formation (46.8 ± 21.3 vs 6.1 ± 2.0; $p<0.01$) and raphe nuclei (83.8 ± 22.1 vs 15.8 ± 4.8; $p<0.001$). Thus, although ineffective in promoting recovery in chronic injuries, DC stimulation does upregulate retrograde axonal transport, as evidenced by increased labelling of neurons by HRP. This may be a mechanism by which DC fields promote recovery after acute injuries.

444.12

HEAD INJURY AND BRAIN ENERGY CONTENT:AN IN VIVO ASSESSMENT BY INTEGRATED MRI/31-P MAGNETIC RESONANCE SPECTROSCOPY. M.Rango*, R.E.Lenkinski*, T.Gennarelli*, W.Alves*, C.McGinnis*, and R.I.Grossman*(SPON:J.Saunders). Dept. of Neurology, Radiology, and Neurosurgery, Univ.of Pennsylvania, Philadelphia, PA 19104.

We have studied the content of brain phosphorus compounds in humans after acute head injury. 13 patients were evaluated at a mean interval of 14 days after trauma and follow-up examination (more than 30 days after injury) was carried out in eight of these cases. Most of the patients were on assisted ventilation. All MRI/MRS studies were performed on a 1.5 T MR Signa scanner equipped with the standard spectroscopic research accessory. Dress localization was employed in eight cases and a 3D-CSI sequence which yielded spectra from 4 cmx4 cmx4 cm voxels in the others. Spectra were processed off-line using a computer program written specifically for the anlysis of in vivo spectra.

In large focal insults the PCr/Pi ratio was reduced in the affected side of the brain. Moreover, in some of these cases little or no observable ATP resonances were detectable. Abnormal spectra in hyperventilated patients exhibited a more normal appearance when the patients were returned to unassisted ventilation. It is not clear, at this point, whether these changes are a reflection of a recovering brain function or are a direct result of the ventilation state of the patient. We are currently extending our studies into the first 48 hours.

AUDITORY SYSTEM

445.1

NON-OLIVOCOCHLEAR CHOLINERGIC PERIOLIVARY CELLS. J.C. Adams, Dept. of Otolaryngology and Comm. Sci., Med. Univ. of S.C., Charleston, S.C. 29425.

A modified Koelle method was used to histochemically localize esterase and in the same brainstem sections of cat, choline acetyltransferase was visualized using immunocytochemistry. This procedure shows that the two enzymes are present in all cells that show the presence of either. Olivocochlear cells show markedly stronger esterase staining than cranial motor nuclei cells. This would be expected based on the assays of Godfrey and his colleagues. The present experiments also show cells that express both cholinergic enzymes but are not olivocochlear cells. These cells are small multipolar cells in the rostral portion of the lateral nucleus of the trapezoid body and have not previously been recognized as a specialized group. Their location and morphology are distinctly different from olivocochlear cells. They also differ by showing very weak esterase staining but show transferase immunostaining that is comparable to that of olivocochlear and cranial motor nuclei cells. Their location in a region where all or nearly all cells project to the cochlear nucleus suggest that these cells may contribute cholinergic descending inputs that nucleus.

445.2

ELECTRICAL STIMULATION OF THE INFERIOR COLLICULUS AT LOW RATES PROTECTS THE COCHLEA FROM AUDITORY DESENSITIZATION. R. Rajan*. (SPON: D.R.F. Irvine). Department of Physiology, University of Western Australia, W.A. 6009, Australia.

Electrical stimulation of the inferior colliculus (IC) contralateral to a cochlea presented a loud sound exposure significantly reduced temporary threshold shifts (TTS) in cochlear sensitivity caused by the exposure. Intra-cochlear perfusion of Hexamethonium blocked this effect, with a time course that paralleled the drug's action on both the classical cochlear effects and the recently-demonstrated TTS effects of electrical stimulation at the brainstem of the efferent pathway, the crossed olivocochlear bundle (COCB). The protective IC effects were obtained at much lower stimulation rates than required with COCB stimulation at the brainstem. Ipsilateral IC stimulation could also protect the cochlea, but only by smaller amounts and at higher rates. Thus the IC appears to provide a strong influence that modulates the excitability levels of the olivocochlear nuclei in the brainstem. Protective effects appear to be exerted by both crossed and uncrossed OCB, and may be due solely to the medial olivocochlear system of efferent fibres that terminate on outer hair cells in the cochlea.

445.3

SHAPE, SIZE, AND ORIENTATION OF THE DENDRITES OF THE COCHLEAR NUCLEUS NEURONS IN GUINEA PIG. L. I. Terr, W. Pimprapaiporn,* and J. K. Moore. Lab. of Neuroanatomy, House Ear Institute, Los Angeles, CA, and Dept. of Anatomical Sciences, SUNY at Stony Brook, NY.

This report provides quantitative and qualitative data on three-dimensional tracing of dendritic trees of the cochlear nucleus neurons and also qualitative 3-D study of them in three guinea pigs. The sections were from tissue blocks stained by the Golgi-Hortega technique and embedded in celloidin. A neuron tracing computer system to reconstruct the shape of the neurons as revealed by metallic impregnation was used. Dendrites and soma contours were traced and entered into a computer with a vector display processor. To characterize the reconstructed neurons as bushy, stellate, giant, granular, or octopus, we evaluated the following: total length of the dendrites; volume of the neurons, including that of the dendrites; cross-sectional area of the soma; number of main dendrite trees originating from the soma; number of the dendritic appendages; and number of branch points. The performed statistical analysis to determine the means and standard deviations of these parameters. We also studied the orientation of the dendrites in relation to the surface of the cochlear nucleus and the root of the cochlear nerve. Findings could be applied to anatomical studies of the pathological changes of dendritic networks in deafness or to physiological studies of the cochlear nucleus. A mathematical neuron model could be made to explore such problems as the relation between the time course and the soma-dendritic location of synaptic input.

445.4

A POPULATION STUDY OF THE COCHLEAR NUCLEUS AND THE AUDITORY NERVE: RESPONSES TO SPEECH SOUNDS. D.O. Kim, S.O. Chang and J.G. Sirianni, Div. Otolaryngol., Dept. Surgery and Neuroscience Program, University of Connecticut Health Center, Farmington, CT 06032

We conducted population studies of neurons of the posteroventral and dorsal cochlear nucleus (PVCN & DCN) and the auditory nerve fibers in decerebrate cats investigating responses to vowels [ɛ] and [a], and pure tones. Our goal is to elucidate how these signals are encoded in the auditory nerve fibers and how they are further processed in the PVCN and DCN. Several types of response profiles are computed: (1) driven discharge rate vs individual neuron's characteristic frequency (CF); (2) d' vs neuron's CF, where d' is a measure of detectability of rate increase a la signal detection theory; (3) spatially-weighted Fourier components of temporal discharge patterns vs neuron's CF a la Young & Sachs' (1979) "ALSR". We find that the response profiles derived from the mean driven rate and d' are generally similar. Our data indicate that the PVCN and DCN neurons' response profiles are different from those of the nerve fibers as follows. The response profiles derived from the driven rate of the primary nerve fibers with high spontaneous rates are severely degraded at 70 dB SPL by saturation of discharge rate limited to about 200 spikes/sec. In contrast, the PVCN and DCN neurons' response profiles are little affected by rate saturation. Some of the PVCN and DCN neurons' discharge rates reached 400-500 spikes/sec at 70 dB SPL. Our observations support the hypothesis that the PVCN and DCN neural mechanisms transform the primary nerve fiber representations of the vowel spectrum into enhanced representations. We postulate that a possible such mechanism is lateral inhibition in the cochlear nucleus. [Supported by a grant R01 NS23693 from NIH and grants from Univ. Conn. Health Center]

445.5

INTERAURAL INTENSITY CODING: AN INTRACELLULAR ANALYSIS USING THE BRAIN SLICE PREPARATION. D. H. Sanes. Depts. Otolaryngol., Physiol. & Biophysics, NYU Med Ctr, New York, NY 10016.

One way in which animals localize sounds in the azimuth is the intensity differences at the two ears. Neurons in the lateral superior olive (LSO) encode this cue by integrating the synaptic drive from ipsilateral excitatory and contralateral inhibitory connections. This synaptic integration has been analyzed in 500 um brain slices through the gerbil superior olive. Intracellular recordings from LSO neurons were observed following the application of independent or conjoint electrical stimuli to the excitatory afferent and inhibitory afferent pathways. Stimulation of ascending fibers from the ipsilateral cochlear nucleus reliably evoked EPSPs and action potentials. Stimulation of the medial nucleus of the trapezoid body (MNTB) consistently evoked IPSPs. MNTB stimulation suppressed synaptically evoked action potentials. When stimulus amplitude was increased to the excitatory pathway, it was generally found that a greater MNTB stimulus was necessary to suppress the action potential. These results confirm that the LSO integrates evoked EPSPs and IPSPs to encode interaural intensity.

445.6

SELECTIVE LOSS OF GABA NEUROTRANSMISSION IN THE INFERIOR COLLICULUS OF AGED FISCHER-344 RATS. A. Raza* and S.P. Arneric (SPON: W.H. Cline), Department of Pharmacology, Southern IL. University School of Medicine, Springfield, IL 62702.

Immunocytochemical studies have suggested an age-related loss of GABA-containing cells in the central nucleus of the inferior colliculus (ICC), a critical auditory nucleus (Caspary & Lawhorn, Neurosci. Abst.13:150.7, 1987). This study sought to determine: 1) Is there an age-related, specific decrease in Ca^{2+}-dependent release of GABA in the ICC? 2) If so, is the GABA impairment regionally specific? Amino acid concentrations were determined by HPLC. Release of endogenous GABA, glutamate (Glu), aspartate (Asp) and ^{3}H-acetylcholine (^{3}H-ACh) was measured from micropunches (1.5 mm dia x 0.5 mm thickness) of the ICC and compared with rostral ventrolateral medulla (RVL) and somatosensory cortex (SSCx) in young (3-7 mo.) and aged (24-26 mo.) Fischer-344 rats. GABA neurotransmission is involved in processing sensory information in these regions. Depolarization with 35 mM K^+-evoked release of GABA (200% of basal), Glu (215%), Asp (163%) and ^{3}H-ACh (309%) which was substantially Ca^{2+}-dependent ($n = 12$, $p < 0.05$). Basal and K^+-evoked release of GABA in the ICC of aged rats was reduced to 32-43% of young, ($n = 8$, $p < .05$), while other transmitters were unaffected. In addition, there was a corresponding 30% reduction in tissue levels of GABA ($p < .05$) while other amino acids were unaffected. This age-related GABA deficit was not observed in RVL or SSCx. CONCLUSIONS: 1) These data support the idea that GABA, Glu, Asp and ACh serve a neurotransmitter role in the ICC; 2) These results support and extend the finding of Caspary & Lawhorn (1987) that GABA neurotransmission is selectively impaired in ICC of aged Fischer-344 rats; 3) Loss of inhibitory GABA neurotransmission in ICC may be one of the neurochemical correlates to the deficits in auditory perception associated with presbycusis. (Supported by the Central Research Committee, Southern IL Univ. School of Medicine and NIA N01-AG-2104)

445.7

DIFFERENTIAL GABAERGIC INFLUENCE ON THE ORIGINS OF THE CORTICAL AND SUBCORTICAL PROJECTIONS OF THE PRIMITIVE MEDIAL GENICULATE. S. B. Frost*, B. N. Baker* and R. B. Masterton (SPON: J. C. Smith). Dept. Psychology, Florida State Univ., Tallahassee, FL 32306.

Previous experiments using retrograde degeneration and orthograde and retrograde axonal transport methods have shown that in a very neurologically primitive mammal, Monodelphis domestica, less than 15% of the cells in the MG project to neocortex while 67% to 80% project to lateral amygdala and putamen. The dominant subcortical projection originates in the caudal 2/3 of MG, while the cortical projection originates in the rostral 1/3.

Conventional receptor binding techniques show that the density of GABA-A receptors in MG parallels this caudal-rostral difference in subcortical projections while mACh and glycine receptors do not. However, anti-GABA immunocytochemistry reveals very few (<1%) GABAergic neurons in MG and these are scattered throughout its rostral-caudal extent. This mismatch of intrinsic GABAergic neurons and GABA-A receptors suggests that most of the GABA-A receptors in caudal MG, those modulating the subcortical pathway, may be accommodating extrinsic sources of GABAergic input.
Supported by NIH-NINCDS # NS7726.

445.8

GLYCINERGIC AND GABAERGIC AUDITORY BRAIN STEM NEURONS AND AXONS IN THE MUSTACHE BAT. G.D. Pollak and J.A. Winer, Dept. of Zoology, University of Texas, Austin, TX 78712, and Dept. of Molecular and Cell Biology, University of California, Berkeley, CA 94720.

Neurochemically distinct pathways were studied in the bat, *Pteronotus parnellii*, with antisera directed against glycine conjugated to glutaraldehyde or glutamic acid decarboxylase (GAD) or gamma aminobutyric acid (GABA) to identify neurons and axon terminals (puncta). There are four main findings. The first is that the medial nucleus of the trapezoid body (MNTB) has predominantly Gl+ neurons. The second finding is that there is a pronounced gradient of increasing immunoreactivity along the caudal-to-rostral axis of MNTB, i.e., along an isofrequency contour. There is also a gradient of Gl+ axons and puncta along the rostro-caudal axis of the lateral superior olive (LSO). Since the LSO receives inhibitory input from the MNTB, it is possible that these gradients establish an orderly distribution of inhibitory thresholds of E-I neurons in LSO. If so, this may be the origin of the inhibitory gradients observed for E-I neurons in the 60 kHz contour of the mustache bat's inferior colliculus. The third finding is that several of the principal auditory nuclei are characterized by the predominance of their immunoreactivity. The MNTB is predominantly Gl+, whereas the LSO and dorsal nucleus of the lateral lemniscus are predominantly GABA+, as are inferior colliculus cells. The fourth finding is that distinct Gl+ and GABA+ axonal fascicles can be traced from the trapezoid body into the 60 kHz contour of the inferior colliculus. This suggests that parallel, putatively inhibitory glycinergic and GABAergic pathways converge within the central nucleus of the inferior colliculus.
Supported by RO1 NS 27286-06 (GDP) and RO1 NS16832-09 (J.A.W.). We thank R. Wenthold for antisera to glycine and GABA.

445.9

ORTHOGONAL TOPOGRAPHICAL REPRESENTATION OF CHARACTERISTIC AND BEST MODULATION FREQUENCY IN THE INFERIOR COLLICULUS OF CAT. G. Langner and C. E. Schreiner*. Zool.Inst.,THD, Schnittspahnstr.3 ,61 Darmstadt, FRG; *Coleman Lab., UCSF, San Francisco, USA

Three-dimensional reconstruction of the location of single and multiunit recordings revealed that the 30-40 neuronal laminae in the central nucleus of the inferior colliculus (ICC) do not -as often assumed- correspond to isofrequency planes with all units most sensitive to about the same characteristic frequency (CF). Instead CF increases roughly from medial to lateral on a given lamina by 10-16%, indicating a tonotopic fine structure.

In addition units in the ICC are tuned to temporal envelope modulations (BMF) up to 1000Hz (Langner,G. and Schreiner, C.E, *Neurophys*. 60: 1799, 1988). BMF was also found to be mapped in "isofrequency planes" with highest BMFs at the lateral-caudal border of the ICC (Schreiner,C. and Langner,G. *Neurophys*. 60: 1823, 1988).

New results verify these findings and indicate that CF and BMF are represented orthogonally: along each line of constant CF on a frequency-band lamina neurons seem to be tuned to a range of BMFs. Finally, response maps obtained by stimulation with a harmonic complex support the conclusion that these neurons are involved in coding periodicity pitch. These findings do not support a 'central spectrum' hypothesis as required for pattern models of pitch perception. *Supported by DFG, SFB 45.*

445.10

INHIBITION SHAPES RESPONSES TO INTERAURAL LEVEL DIFFERENCES IN THE INFERIOR COLLICULUS OF THE BARN OWL.

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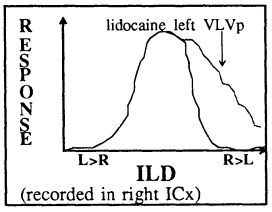
Terry T. Takahashi, Institute of Neuroscience, University of Oregon, OR 97403.

Barn owls use interaural differences in sound pressure level (ILD) to compute location of the source in elevation. The external nucleus of the inferior colliculus (ICx) maps auditory space. Elevationally restricted auditory receptive fields are coded for by non-monotonic neuronal responses to ILD.

ICx obtains polysynaptic input from lower brainstem nuclei. Information about ILD is carried in an anatomically and functionally distinct pathway into ICx. Important lower brainstem nuclei of the ILD stream include nucleus angularis and nucleus ventralis lemnisci lateralis, pars posterior (VLVp).

We injected the local anesthetic lidocaine hydrochloride focally into VLVp while recording neuronal responses to varying ILD in the contralateral ICx. VLVp was found to contribute functional inhibition at ILDs favoring the ear ipsilateral to the side on which responses were recorded in ICx (shown schematically in the figure). This result is consistent with the known hodology and with the functional response properties of VLVp. Preliminary results support the hypothesis that this inhibition is established directly by VLVp in the lateral shell of the central nucleus of the inferior colliculus.



R.A. is a Howard Hughes Medical Institute Fellow.

445.11

DYNAMIC CHANGES IN RECEPTIVE FIELDS OF BARN OWL AUDITORY-MIDBRAIN NEURONS. H. Wagner. Max-Planck-Institut für biologische Kybernetik, Spemannstrasse 38, D-7400 Tübingen, FRG

The receptive field has been a fruitful concept for characterizing the response of neurons in various stimulus dimensions. Auditory neurons in the barn owl's inferior colliculus have spatial receptive fields (Knudsen E.I. and M. Konishi, *J. Neurophysiol.* 41: 870 (1978)). The deviation of a sound source from the midsagittal plane, azimuth, is coded by the interaural time difference (ITD). I analysed the temporal properties of the ITD- and frequency tuning of these neurons, a hitherto neglected aspect of their response characteristics.

Standard extracellular recording techniques were used to record the ITD-tuning of 256 neurons and the frequency tuning of 100 neurons to dichotic stimulation with noise or tone bursts of 100 ms duration. The ITD- and frequency selectivity of the neurons in the inferior colliculus is broad first and improves to a final value with time. ITD-selectivity was higher in the interval from 21-40 ms after stimulus onset than in the interval from 1-20 ms in 87% of the neurons. The improvement amounted to a mean value of 40%. An improvement of frequency tuning was found in 67% of the cases.

It is concluded that a) dynamic improvement is due to a process within the network computing ITD- and frequency selectivity, possibly mediated by GABAergic inhibition (Fujita and Konishi, *Soc. Neurosci. Abstr.* 14: 1096 (1988)), b) the spatial tuning of single neurons is higher than previously thought, and c) dynamic improvement may be an adaptation to detect motion acoustically.

445.12

PHYSIOLOGY AND TOPOGRAPHY OF NEURONS WITH MULTIPEAKED TUNING CURVES IN CAT PRIMARY AUDITORY CORTEX. M.L. Sutter* and C.E. Schreiner (SPON. P.Leake) Coleman Laboratory and Bioengineering Group, University of California, San Francisco CA 94143-0526.

In previous studies, multiple-unit recordings revealed a systematic change of the sharpness of tuning along the iso-frequency domain of cat primary auditory cortex (AI).The most sharply tuned unit clusters were found in the central 2 mm of iso-frequency stripes. Approaching the dorsal and ventral limits of AI, neuronal responses became progressively more broadly tuned. This study investigated the properties and distribution of single units with multipeaked frequency tuning curves (FTCs) relative to the observed gradient of sharpness of tuning obtained for multiple unit responses. Single unit locations were roughly classified as 'central' (located within 2 mm of the area containing the most sharply tuned responses of AI), 'dorsal', or 'ventral'. In the dorsal area, 35% of encountered single units had multipeaked FTCs, i.e., FTCs with more than one distinct threshold minimum. In the central area, 3% of the studied neurons had multipeaked FTCs. No multipeaked units were encountered in the ventral area.

Eighty percent of the studied multipeaked FTCs had two peaks. The frequency separation of minima in multipeaked FTCs ranged from 0.36 to 2.8 octaves (mean separation: 0.77±0.5 octs.). Strong inhibition was often found between adjacent peaks. Disparate response latencies as great as 19 ms could be seen between two peaks of the same unit. Response latencies 30 dB above threshold were 22±8 ms. It is concluded that neurons with multipeaked FTCs represent a physiologically distinct neuron response subclass in the dorsal AI. Research supported by the Coleman Fund and the NIH Training Grant GM 8155.

INVERTEBRATE LEARNING AND BEHAVIOR I

446.1

INHIBITORY CONTROL OF IDENTIFIED SEROTONERGIC NEURONS IN THE LOBSTER. P.M.Ma*, W.A.Weiger*, and E.A.Kravitz. Department of Neurobiology, Harvard Medical School, Boston, MA 02115.

The injection of serotonin into freely moving lobsters produces a posture resembling that seen in dominant animals in a hierarchy; injection of octopamine produces an opposing posture resembling that of subordinate animals. We have been examining the role of amines in the production of these postures by intracellular recording from identified aminergic neurons in the lobster ventral nerve cord. Immunocytochemical methods have identified over 100 serotonergic neurons in the lobster central nervous system. Our current focus is on two pairs of large serotonergic neurons, one each in the first abdominal (A1) and fifth thoracic (T5) ganglia. These cells are spontaneously active and their rate of firing is regulated both by an endogenous oscillatory mechanism and by external synaptic input. In other systems, inhibition appears to be important in suppressing certain behaviors, while removal of inhibition results in the emergence of new behaviors. Therefore, our recent efforts have centered on identifying the nature of the inhibitory inputs to the A1 serotonin cells. Three neurotransmitters inhibit these cells: GABA, octopamine, and serotonin. GABA mediates a rapid, short-term inhibition of the A1 serotonin cells; its role will be addressed more fully in the next abstract. Exogenous octopamine reversibly decreases the firing rate of the A1 serotonin cells while phentolamine, an octopamine blocker, increases the firing rate. The latter result suggests the presence of tonic octopaminergic inhibition *in vivo*. Octopamine acts independently of the GABAergic inhibitory system, and its effects appear to be mediated by a depletable second messenger. Exogenous serotonin slows the firing rate of the A1 serotonin cells, indicating a possible autoinhibition of the cells by released serotonin. Inhibitory control of these cells therefore appears to be a complex process involving rapidly acting short-term as well as slowly acting long-term components. (Supported by NIH.)

446.2

GABAERGIC INHIBITION OF IDENTIFIED LOBSTER SEROTONERGIC NEURONS. W.A.Weiger*, P.M.Ma*, and E.A.Kravitz (SPON: M.K.Worden). Department of Neurobiology, Harvard Medical School, Boston, MA 02115.

Inhibition plays an important role in the control of behavior. Results in several systems suggest that inhibitory neurons suppress certain behaviors, and a decrease in activity of such neurons may release previously inhibited behaviors. Our recent efforts have focused on the nature of the inhibitory synaptic input to the pair of large serotonin cells in the first abdominal (A1) ganglion of the lobster. These A1 serotonin cells promote the adoption of a posture which resembles that seen in dominant animals in a hierarchy. In the previous abstract, three neurotransmitters were identified as inhibitory to these cells. Two of these, octopamine and serotonin, were discussed in the previous abstract; the third, GABA, will be the subject of the current abstract. Exogenously applied GABA reversibly slows the firing rate of the A1 serotonin cells, even in the presence of low Ca^{++}/high Mg^{++} saline. Picrotoxin, a GABA antagonist, acts in a reversible manner to cause the disappearance of all inhibitory post-synaptic potentials (IPSP's) recorded from the A1 serotonin cell somata and to produce a large increase in the firing rate of the cells. These data suggest that GABAergic neurons provide direct inhibitory inputs to the A1 serotonin cells and account for the IPSP's which can be detected in the soma. Lesion studies suggest that neurons located in the third abdominal (A3) ganglion are responsible for the production of most of the IPSP's seen in the A1 serotonin cells. These A3 neurons may be GABAergic inhibitory neurons or excitatory neurons which act through GABAergic inhibitory interneurons in the A1 ganglion. In our future work, we plan to find and record from the A3 inhibitory neurons and to investigate the inhibitory inputs to the A1 serotonin cells in lobsters of known position in a dominance hierarchy. We will test the idea that greater inhibition of these cells is found in subordinate lobsters, and that in dominant lobsters these cells show a lesser degree of inhibition. (Supported by NIH.)

446.3

CHARACTERIZATION OF SPECIFIC EARLY PROTEINS INDUCED BY 5-HT AND cAMP DURING ACQUISTION PHASE OF LONG-TERM FACILITATION IN *APLYSIA* SENSORY NEURONS. A. Barzilai, T.E. Kennedy, J. D. Sweatt and E. R. Kandel. HHMI, Columbia, N. Y., N.Y. 10032.

Induction of long-term memory for sensitization and long-term presynaptic facilitation by 5-HT and cAMP in sensory neurons is dependent on active transcription and translation during the acquisition phase, the 1.5-hour period during which 5-HT is present. To gain insight into these long-term events, we have focused initially on the earliest detected transcriptional changes induced by 5-HT and cAMP. Analysis of ^{35}S-methionine incorporation into proteins using analytical 2-D gels indicate that 5-HT rapidly induces transcriptionally-dependent changes in 15 proteins, within 15 to 30 minutes. These changes are transient, and subside within 1 to 3 hours. The same proteins were also induced by cAMP, suggesting that the induced genes might bear a cAMP recognition element. In these several features -- rapid and transient induction, transcriptional dependence, and requirement for a second messenger -- these proteins resemble the immediate early gene products induced in vertebrate cells by growth factors. To test this idea and to explore the regulatory and effector roles of these proteins in the induction of long-term sensitization, we have obtained partial amino acid sequence of three *Aplysia* early proteins using the method of Kennedy et al. (*Proc. Natl. Acad. Sci. USA*, 1988), and are attempting to obtain cDNA clones corresponding to the proteins.

446.4

A PROTEIN IN *APLYSIA* SENSORY NEURONS THAT INCREASES IN BOTH LEVEL OF PHOSPHORYLATION AND RATE OF SYNTHESIS DURING FACILITATION. K. Wager-Smith*, A. Barzilai, J.-F. Brunet*, E.R. Kandel, J.D. Sweatt. Columbia Univ. NY.,NY.

Short-term facilitation (STF) of *Aplysia* sensory-motor synapses (as induced by a 2 minute application of 5-HT) differs from long-term (24 hour) facilitation (LTF) (as induced by a two hour application of 5-HT) in that LTF requires protein and RNA synthesis. To study the basis of this macromolecular synthesis dependence we are characterizing proteins that have been shown by Barzilai et al. (*Neuron*, 1989) to change in their rate of synthesis during the acquisition phase of LTF. One of the first proteins to go up in synthesis (or a protein that migrates to the same spot on 2D gels: pI = 5.0, M.W. = 28 KD) also increases in its level of phosphorylation after two hours of 5-HT treatment. This delayed phosphorylation increase is distinct from the previously described, more rapid phosphorylation changes that occur in 17 other proteins (Sweatt and Kandel, *Nature*, 1989). Preliminary subcellular fractionation studies suggest that this protein is present in the nucleus. Perhaps this protein contributes to the switch from STF to LTF by detecting the 5-HT-stimulated kinase activity at a critical period after the protein's synthesis. Alternatively, the increase in phosphorylation might simply reflect the increase in the protein's mass. To further characterize this protein, we have obtained partial amino acid sequence and are now attempting to clone the corresponding cDNA.

446.5

CHARACTERIZATION OF CHANGES IN LATE PROTEIN AND mRNA EXPRESSION DURING THE MAINTENANCE PHASE OF LONG-TERM SENSITIZATION. T.E. Kennedy, D. Kuhl*, A. Barzilai, E.R. Kandel, and J.D. Sweatt. HHMI and Columbia Univ., NY, NY 10032.

Maintenance of long-term sensitization in *Aplysia* is associated with a sustained increase in the incorporation of ^{35}S-methionine into four proteins. Two of these proteins, #407 and #1603, show a similar change of expression in isolated clusters of pleural sensory neurons when 5-HT, rather than tail shock, is used to produce long-term facilitation. The expression of these proteins is blocked by inhibitors of macromolecular synthesis present during the period of 5-HT application. We have cloned the cDNA corresponding to protein #407 using partial amino acid sequence, and found no significant homologies in sequence data bases. By contrast, partial amino acid sequence, pI and M.W., indicate that protein #1603 is the *Aplysia* homologue of GRP78/BiP. *Aplysia* BiP also changes its expression in pleural sensory neuron clusters following application of cAMP or heat shock to 37° C. BiP is thought to function as a chaperon of newly synthesized proteins in the lumen of the E.R. This is consistent with the finding that *Aplysia* BiP first increases in expression at 3 hours following 5-HT application when overall protein synthesis is maximal (Barzilai et al., 1989). Using PCR, we have obtained a partial cDNA clone of *Aplysia* BiP and are examining the distribution and expression of both of the late proteins following long-term training.

446.6

PERSISTENT LOSS OF REGULATORY SUBUNITS OF THE cAMP-DEPENDENT PROTEIN KINASE IN APLYSIA SENSORY NEURONS REQUIRES SYNTHESIS OF NEW PROTEIN DURING THE CRITICAL PERIOD FOR THE INITIATION OF LONG-TERM PRESYNAPTIC FACILITATION (LTF) P.J. Bergold, J. D. Sweatt, E. R. Kandel, and J. H. Schwartz. Center for Neurobiology & Behavior and HHMI, Columbia University, NY 10032

Initiation of LTF in Aplysia has a critical period for protein synthesis during application of 5-HT. cAMP-dependent protein kinase, a heterodimer of regulatory (R) and catalytic (C) subunits, is activated in both short- and long-term facilitation. Presumably this explains the persistent cAMP-dependent phosphorylation seen during LTF that is dependent on protein synthesis (Sweatt and Kandel, Nature, 1989). In sensory cells of long-term sensitized animals, the amount of R is reduced with no change in C (Greenberg, et.al., Nature, 1987). To relate these observations we determined if the change in the R-to-C ratio is also dependent on protein synthesis. Treatment with 5-HT resulting in LTF changes the ratio of R-to-C 24 h later. Inhibition of protein synthesis during the treatment blocked the loss of R while inhibition of protein synthesis immediately after had no effect. Elevation of intracellular cAMP is sufficient to induce LTF. R is reduced 24 h after treatment with a cAMP analog plus phosphodiesterase inhibitor. Elevation of cAMP by 5-HT changes the ratio of R-to-C, and this is a new mechanism for long-term regulation of any protein kinase. The change in R may be transcriptional or due to increased turnover. cDNAs encoding Aplysia R and C have been isolated (Beushausen, et. al., Neuron, 1988; Bergold et. al., Neurosci.Abs., 1988) to test if the loss of R is transcriptional. We are also obtaining antisera against R to measure its turnover.

446.7

THE PKC CYCLE: A MECHANISM FOR LONG-TERM AUTO-ACTIVATION OF PROTEIN KINASE C. T.C. Sacktor, A. Calignano and J.H. Schwartz. HHMI, Ctr. for Neurobiol. & Behav., Columbia Univ., NY 10032

Aplysia neurons have two forms of PKC that can be distinguished by their response to arachidonic acid (AA). One is activated at high concentrations of AA (similar to vertebrate β), the other at low concentrations (analogous to γ). In Aplysia neurons, facilitatory stimuli (5-HT or phorbol ester) activate PKC, translocating the kinase from cytosol to membrane. On the membrane, lipokinin, a M_r 29,000 protein (Calignano, Piomelli, Wallner & Schwartz, Neurosci. Abstr. 1988, 14:131), when phosphorylated by PKC through either phorbol ester or 5-HT, activates PLA_2 to produce AA. AA continues to stimulate PKC after the transient second-messenger signal for the initial translocation is dissipated, thereby maintaining the kinase in an active form to enhance synaptic strength persistently: the cycle is thus completed and continued. This mechanism for persistence is relevant for models of learning and memory where PKC plays an important role: LTP in hippocampus, conditioning of the rabbit eye-blink response, and sensitization of defensive reflexes in Aplysia.

446.8

MEMORY MUTATIONS AFFECT AN ELEMENTARY EXPERIENCE-DEPENDENT MODIFICATION OF RESPONSE IN AN IDENTIFIED SENSORY NEURON OF DROSOPHILA. G. Corfas* and Y. Dudai. Dept. of Neurobiology, Weizmann Institute, Rehovot 76100, Israel.

Stimulation of the thoracic bristles of Drosophila elicits a cleaning reflex. The habituation of this reflex is defective in two memory mutants: rut, which lacks Ca^{2+}/calmodulin-activated adenylate cyclase, and dnc, which has a reduced cAMP phosphodiesterase (PDE) activity. We have now investigated the physiology of a sensory neuron that subserves the cleaning reflex. Extracellular recordings were performed from truncated bristles. The recording electrode was also used for mechanical stimulation. We have studied two plastic processes in the sensory neuron: the decrement of response to a sustained stimulus (adaptation), and the decrement of response to repetitive stimuli (sensory fatigue). The mutations rut and dnc did not affect adaptation but did alter sensory fatigue. rut mechanosensory neurons fatigued slower and dnc neurons fatigued faster than normal. The opposite effect of the rut and dnc mutations on sensory fatigue suggested that this process is cAMP dependent. To test this hypothesis we investigated the effect on sensory fatigue of systemic injection of drugs which affect the cAMP cascade. PDE inhibitors caused rapid fatigue in wild-type (CS) neurons, mimicking the effect of dnc. In contrast, rut neurons were not affected by PDE inhibitors. The adenylate cyclase activator forskolin decreased sensory response in naive CS and rut neurons. The protein kinase inhibitor H7 reduced the effect of repetitive stimulation in CS and dnc neurons. Our results indicate: a. the cAMP cascade is involved in sensory fatigue; b. memory mutations affect an elementary form of neuronal plasticity.

446.9

PKC ACTIVATION IS NECESSARY FOR BOTH SHORT- AND LONG-TERM LEARNING-PRODUCED EXCITABILITY CHANGES IN HERMISSENDA TYPE B CELLS. Schuman, Erin M. and Farley, J. Program in Neural Science, Indiana University, Bloomington, IN 47405

Introduction of the PKC inhibitor H-7 into Type B cells prior to in vitro conditioning resulted in a failure of these cells to exhibit the pairing-specific neural changes typical of controls: cumulative depolarization and increased input resistances. In contrast, inhibition of Ca^{2+}/CAM-dependent PK's before conditioning (via W-7 ionophoresis or bath application) had no decremental effect on the changes. Voltage-clamp analysis of ionic currents following in vitro conditioning revealed that H-7 pre-treatment also prevented the conditioning-produced reduction of I_A.

Comparison of I_A magnitudes in the presence or absence of H-7, 24-48 hrs. following standard behavioral training of intact animals, revealed pairing specific reductions in I_A only when recorded in the absence of H-7. I_A from trained animals recorded in the presence of H-7 was not significantly different from those of untrained animals recorded in normal ASW. These results suggest that persistent activation of PKC is necessary for the expression of plasticity during long-term retention. We are now undertaking studies to investigate whether similar differences exist for $I_{K\text{-}Ca}$.

446.10

SELF-TUNING BY A NEURAL CIRCUIT: THE DSI NEURONS OF THE TRITONIA ESCAPE SWIM CENTRAL PATTERN GENERATOR BOTH MODULATE AND MEDIATE CONNECTIONS IN THE SWIM CIRCUIT. W.N. Frost and P.A. Getting. Dept. Physiology & Biophysics, Univ. of Iowa Iowa City, IA 52242.

The marine mollusk Tritonia responds to noxious stimuli with an escape behavior consisting of a series of vigorous ventral and dorsal body flexions. This escape swim is mediated by an identified central pattern generator consisting of dorsal swim interneurons (DSIs), ventral swim interneurons (VSIs) and the interneuron C2. These interneurons also drive the motor elements of the circuit, the ventral flexion neurons (VFNs) and the dorsal flexion neurons (DFNs).

The central pattern generator interneuron C2 makes connections onto all of the circuit interneurons and flexion neurons. This study focussed on the C2 to DFN-A synapse, one of the output connections from the central pattern generator. We report here that the DSIs, which have previously been shown to contain serotonin, have a powerful modulatory influence on this connection. In high divalent sea water, DSI trains (5-7 Hz, 15sec) produced an average 3.3 fold amplification of the C2 to DFN-A monosynaptic EPSP (N=9, p<.001). This modulation developed within five seconds of the DSI train onset and disappeared within thirty seconds of the train offset. Since the DSIs fire at the onset and then phasically throughout the swim, this modulation should be invoked during the swim.

This finding indicates that the DSI neurons not only participate in mediating the oscillatory central pattern for the swim, but also modulate the strength with which this pattern is coupled to the circuit flexion neurons. This suggests that in the initial seconds of the escape response the circuit may actively "tune" itself into an effective state for swimming. We are now exploring the degree to which this tuning process extends to connections made by C2 within the central pattern generator itself.

Supported by NIH grants NS17325 & NS07247.

446.11

ANALYSIS OF NON-ASSOCIATIVE LEARNING IN *C. ELEGANS* : I NEURAL CIRCUIT MUTATIONS. Catharine H. Rankin and Martin Chalfie. Department of Psychology, Univ. of British Columbia, Vancouver, B.C. V6T 1Y7 and Department of Biological Sciences, Columbia Univ. N.Y., N.Y. 10027.

A major advantage of *C. elegans* as a model system for the study of learning and memory is the wealth of genetic information available. Genetic analysis has played a major role in elucidating the neuroanatomical circuit underlying the touch reflex in this animal. A variation of this reflex, the reversal response to a vibrational stimulus produced by tapping the side of the holding dish, has been shown to be capable of habituation, dishabituation, sensitization and inhibition. We are now in the position to use the existing information about the neural circuit to determine the neural pathways involved in learning and to use learning to probe the interaction between various elements of the neural circuit.

An example of this type of analysis was determining whether the circuit underlying the reversal response was the same as the circuit for head touch. A mutant (mec-3) with defective primary touch receptor cells (the microtubule cells) did not reverse to taps; thus, the same sensory receptors transduce both head touch and taps. We have also examined whether the known neural pathway for tail touch also underlies the tail touch induced inhibition of reversal to tap. Two tail touch insensitive mutants failed to show inhibition. One mutant (Egl-5) showed that the same sensory receptors were necessary for both responses to tail touch and inhibition. Another mutant (Deg-1) showed that the primary interneuron (PVC) in the posterior tail touch circuit is necessary for inhibition. These types of experiments will allow us to determine the neural circuit involved in the learning as well as potential sites of plasticity.

446.12

ANALYSIS OF NON-ASSOCIATIVE LEARNING IN *C. ELEGANS*: II DEVELOPMENT OF BEHAVIOR AND PLASTICITY.
C. D. O. Beck*, C. M. Chiba* and C. H. Rankin (SPON: R. Lane). Dept. of Psychology, Univ. of British Columbia, Vancouver, B.C. V6T 1Y7.

The touch circuit of *Caenorhabditis elegans* provides a unique opportunity to examine the relationship between known neuroanatomical development and development of learning. Using vibrational stimuli (taps to the side of the holding dish) we have demonstrated that *C. elegans* is capable of all forms of non-associative learning. Before analyzing the development of non-associative learning, the ontogenetic changes in the behavioral elements of the touch reflex were examined in the four larval stages and two adult stages (young adults and 4-day old adults). The frequency of spontaneous reversals was significantly higher in young adults than any other stage ($F(5,54)= 4.84$, $p=.001$). Although animals at all stages respond to a head touch by reversing direction, adult stages were significantly more likely to reverse to a tap than were the larval stages ($F(5,114)=13.18$, $p=.0001$). Larval stages were more likely to accelerate forward in response to the tap; this became more pronounced as stimulus intensity increased. The behavioral changes between the last larval stage and young adulthood correlate with known neuroanatomical changes.

Despite these developmental changes, animals from all stages exhibit habituation and inhibition of the reversal response. A detailed analysis of the parameters of these forms of learning will indicate whether they have the same characteristics as observed in adult animals.

CIRCUITRY AND PATTERN GENERATION

447.1

MULTISECOND AFTERDISCHARGE OF SPINAL CORD INTERNEURONS EVOKED BY CUTANEOUS STIMULATION IN THE ROSTRAL SCRATCH RECEPTIVE FIELD OF THE TURTLE.
Scott N. Currie and Paul S.G. Stein. Dept. of Biology, Washington University, St. Louis, MO 63130.

Stimulation of cutaneous afferents in the turtle increases the excitability of scratch reflex for several seconds following stimulus-offset (J. Neurophysiol. 60: 2122-2137, 1988). In the present study, we recorded from single cutaneous afferents and sensory interneurons in order to examine the neuronal mechanisms for multisecond increases in scratch excitability. A single segment of the midbody spinal cord was isolated in situ by transecting the cord at the segment's anterior and posterior borders. The isolated segment was left attached to its peripheral nerve that innervates part of the rostral scratch receptive field. A micro-suction electrode (5 µm I.D.) was used to record from descending axons in the white matter. We identified a subset of sensory interneurons that exhibited strong afterdischarge for as long as 10-30 seconds following a mechanical or electrical stimulus to the rostral scratch receptive field. These cells displayed robust temporal summation in response to single electrical pulses delivered to the shell at multisecond intervals. Cutaneous interneurons with long afterdischarge may serve as a neural locus for multisecond excitability changes in the spinal network for scratch reflex. Supported by NIH Grants NS07057 to S.N.C. and NS15049 to P.S.G.S.

447.2

A PERIPHERAL FEEDBACK LOOP MEDIATED BY NEUROMODULATORY SEROTONERGIC AFFERENTS COUPLES TWO CPGs IN A PHASE-INDEPENDENT FASHION. P.S. Katz and R.M. Harris-Warrick. Section of Neurobiology and Behavior, Cornell University, Ithaca, NY 14853.

In the foregut of the crab, *Cancer borealis*, serotonergic mechanosensory afferents, called GPR cells, are activated by movements of the gastric mill, but not by movements of the faster pyloric filter (Katz and Harris-Warrick, J. Neurophys. in press). However, these cells have slow neuromodulatory effects on neurons in the pyloric central pattern generator of the stomatogastric ganglion. The GPR cells cause prolonged enhancement of bursting in the PD/AB pacemaker cell group, excite the LP neuron, and inhibit the PY neurons. Some of these effects last only during the GPR stimulation, while others slowly decline over many cycles. GPR input alters the phase relationships between pyloric neurons by changing the relative levels of excitation of the pyloric cells in a manner that is mimicked by serotonin application. The effects of GPR stimulation on these cells are not mediated by discrete fast synaptic potentials and are therefore independent of the phase of the pyloric cycle at which the input occurs. Because the GPR cells are rhythmically active in time with the gastric mill, their effects on the pyloric pattern occur rhythmically with each gastric mill cycle. Thus, the slow neuromodulatory effects of these mechanosensory afferents coordinate the pyloric pattern to the gastric mill cycle through a mechanism that does not require the input to be precisely timed to the pyloric pattern. Supported by NIH NS17323 and Hatch Act Grant 191410.

447.3

TEMPERATURE DEPENDENCE OF GRADED CHEMICAL SYNAPTIC STRENGTH WITHIN THE PYLORIC MOTOR CIRCUIT OF THE LOBSTER STOMATOGASTRIC GANGLION.
B. R. Johnson and R. M. Harris-Warrick. Neurobiology and Behavior, Cornell University, Ithaca, NY 14853.

Spiking cells of the central pattern generator (CPG) for the pyloric motor pattern in the stomatogastric ganglion (STG) of the spiny lobster *Panulirus interruptus* use graded synaptic transmission (electrical coupling and chemical inhibition) to coordinate their rhythmic activity. We examined the temperature dependence of graded synaptic transmission between identified pyloric CPG cells in STGs isolated from animals maintained at 15°C. Synaptic input-output curves for graded transmission were gathered in 10-7M TTX by measuring the postsynaptic peak polarizations resulting from 1 sec presynaptic polarizations of varying sign and amplitude. As the temperature was lowered from 23 to 10° C, graded chemical synaptic potentials showed reductions in peak amplitudes of approximately 55 to 100% between different cell pairs. These reductions in graded chemical synaptic strength were not accounted for by general membrane resistance decreases; they were accompanied by increased antidromic action potential size in both pre- and postsynaptic cells, increased presynaptic soma input resistance, and increased electrical coupling between some presynaptic cells. These reductions occur within natural temperature ranges for the spiny lobster (12-21°C), suggesting that the balance of synaptic interactions producing this motor rhythm may be a dynamic function of environmental temperature. Supported by NRSA #NS07859 (BRJ) and NIH grant #NS17323 (RMH-W).

447.4

CONTROL OF THE INTERACTIONS OF TWO MOTOR PATTERN GENERATORS BY RED PIGMENT*CONCENTRATING HORMONE.
P.S. Dickinson, C. Mecsas and E. Marder, Depts. of Biology, Bowdoin College, Brunswick, ME 04011 and Brandeis University, Waltham, MA 02554

The stomatogastric nervous system of the spiny lobster, Panulirus interruptus, generates 4 motor patterns, which control the 4 parts of the foregut. A modulatory peptide, red pigment concentrating hormone (RPCH), activates the cardiac sac rhythm and increases the interactions between the cardiac sac rhythm and the gastric rhythm. In the presence of RPCH, with other inputs to the stomatogastric ganglion blocked, the neurons of the gastric pattern generator fire entirely in cardiac sac time. One mechanism which may account for this "take-over" of the gastric rhythm is a selective increase in the size of the synaptic potentials which the IV (inferior ventricular) fibers, of the cardiac sac pattern, generate on many of the gastric mill motor neurons. In the presence of RPCH, these synaptic potentials increase significantly in amplitude, so that they effectively drive the gastric mill neurons. The input impedance of the gastric neurons does not appear to change in RPCH, suggesting that RPCH may act presynaptically on the IV fibers. Supported by NSF BNS-8706568 and NIH NS-17813.

447.5

A NOVEL SLOW RHYTHMIC PATTERN GENERATED BY THE *APLYSIA* BUCCAL GANGLION.
S.L. Hooper, K.R. Weiss, and I. Kupfermann Cntr. Neurobio. & Behav., Columbia U. P&S and NYS Psych. Inst., NYC 10032

Prior work (Hooper et al., Soc. Neurosci. Abst. 14:608) has shown that crop dilation, a known satiation signal, excites certain buccal ganglion neurons. We therefore chronically implanted extracellular electrodes on various buccal nerves to monitor buccal ganglion activity during feeding and satiation. These recordings reveal that the buccal ganglion can produce an unusual rhythmic pattern that has no obvious visible external behavioral correlates. The pattern consists of organized bouts of spiking activity lasting 0.5 to 2 min that repeat every 3 to 10 min. This slow pattern differs from known buccal mass motor patterns (biting, swallowing, rejection) in two ways. First, the spiking activity in these patterns lasts only 5 to 10 sec. Second, these patterns have much shorter periods (during continuous presentation of food or rejection inducing stimuli), repeating every 10 to 20 sec.

The slow pattern is seldom observed in unfed animals, either aroused or unaroused, but is always seen following meals, and can continue for several days after a meal. The slow pattern can also be induced *in vitro* by crop dilation or electrical stimulation of the esophageal nerve (through which crop derived sensory input projects centrally). Preliminary work in semi-intact preparations indicates that the slow pattern induces contractions of (at least) superficial buccal mass muscles, but does not cause radula movements. These observations suggest that the slow pattern is specifically induced by food in the crop and may be involved in some aspect of food processing.

Both the slow pattern and satiation are induced by crop dilation and electrical stimulation of the esophageal nerve. However, we do not believe that the slow pattern and satiation are causally related, as sub-satiating meals induce the slow pattern, and animals that are expressing the slow pattern but are not satiated readily eat when food is again presented.

447.6

AN IDENTIFIED INTERNEURON TERMINATES PATTERNED MOTOR ACTIVITY IN THE BUCCAL GANGLIA OF HELISOMA.
A.D. Murphy, and F. Mehdizadeh-Namin*. Dept. of Biol. Sci., Univ. of Illinois at Chicago, Chicago, IL 60607.

Patterned motor activity (PMA) underlying feeding in the snail, Helisoma, must be regulated by descending inhibition from the "brain" (circumesophageal ganglionic ring). The buccal ganglia (BG) contain most of the interneurons comprising the central pattern generator that mediates feeding. The BG are connected to the brain via the cerebro-buccal connectives (CBCs). Severing the CBCs or applying a procaine block to the CBCs in a semi-intact animal typically activates rhythmic "feeding" movements. To localize putative inhibitory neurons in the brain we did retrograde staining of cut axons in the CBC using Lucifer Yellow. This revealed a neuron with a soma in the pleural ganglion and an axon that traverses the pleuro-pedal connective, the pedo-cerebral connective and the CBC. Subsequent intracellular recordings confirmed that this neuron, PLBI1 (pleuro-buccal inhibitory neuron 1), is a potent inhibitor of PMA in the BG. A train of action potentials in PLBI1 terminates PMA even in the presence of 10^{-6} M serotonin. Serotonin is an excitatory neuromodulator which induces vigorous PMA at that concentration. Intracellular injection of PLBI1 with Lucifer Yellow CH revealed a neuritic arbor in the pleural ganglion and axon branches arborizing in the ipsilateral parietal ganglion, the ipsilateral pedal ganglion, and the ipsilateral cerebral ganglion. The main axon traversed the ipsilateral CBC and innervated both buccal ganglia, crossing the buccal commissure. (supported by NIH Grant 1 RO1 NS26145-01).

447.7

CONNECTIONS OF IDENTIFIED INTERNEURONS IN THE LEECH ARISE IN NEURAL NETWORKS TRAINED BY BACK-PROPAGATION.
S.R. Lockery, G. Wittenberg, W.B. Kristan,Jr. and T.J. Sejnowski. U.C.S.D. and Salk Institute, La Jolla, CA 92037.

Interneurons in model networks generated by the back-propagation algorithm were compared to identified interneurons in the local bending reflex of the leech. Touching dorsal, ventral, or lateral skin causes a localized withdrawal from the stimulus (local bending). Four mechanoreceptors (P cells) with dorsal or ventral receptive fields provide the major input to the reflex. P cells connect to eight types of dorsal and ventral motor neurons via interneurons, of which nine types have been identified. Strengths of P cell to interneuron connections were measured as the peak synaptic potential produced by a standard P cell impulse train. Output connection strengths for two interneurons were measured as the amplitude of synaptic potentials in the motor neurons following fixed depolarization of the interneuron. We trained a model network of four sensory, eight motor, and nine pairs of interneurons using back-propagation to reproduce the amplitudes of synaptic potentials of the motor neurons during the three forms of local bending. The network included known lateral connections among motor neurons, but no constraints were placed on the pattern of interneuronal inputs and outputs. However, following training the model interneurons, like the real ones, received inputs from dorsal and ventral P cells, and connected to all eight motor neurons. Additional biological constraints, including recurrent connections and temporal response properties, can now be used to improve the predictions of the model.

Supported by NIH research grant NS25916 and the Bank of America-Giannini Foundation.

447.8

INTERSEGMENTAL INTERNEURONS CONTRIBUTE TO MULTIPLE BEHAVIORS IN THE LEECH .
G. Wittenberg, S. R. Lockery, and W. B. Kristan, Jr. Dept. of Biology, Univ. of California at San Diego, La Jolla, CA 92093-0322.

Electrophysiological studies in the CNS of the leech, *Hirudo medicinalis*, were performed in order to define and understand the production of particular behaviors. When the leech receives a mechanosensory stimulus, it produces one of several types of motor patterns, three of which are local bending, swimming, and shortening. Local bending is a segmental withdrawal response produced by excitation of motor neurons (MNs) innervating longitudinal muscles on the side stimulated and by inhibition of MNs innervating muscles on the opposite side. Swimming involves the entire body and is produced by antiphasic rhythmic excitation and inhibition of the same longitudinal MNs. Shortening is a graded response, involving from a few to all segments anterior and posterior to the site of stimulation; shortening is produced by excitation of all longitudinal MNs. More than one interneuron (IN) contributes to shortening as unilateral lesions to the interganglionic connectives cause only a partial decrement in the response of segments distal to the lesion. INs which mediate local bending and swimming behavior have been identified and characterized. We have used intracellular recording and stimulation of these and other INs in an isolated CNS to begin to study their responses and effects in shortening behavior.

Local bending INs and swim INs both contribute to shortening behavior. Cell 115, which participates both in local bending and swimming, produces excitation of dorsal longitudinal MN two segments posterior to the segment in which it receives both dorsal and ventral sensory inputs. Cell 208, a swim IN, has the same motor effect, but receives a highly variable sensory input. In addition, previously unidentified INs participate in shortening. One of these causes excitation of dorsal MNs, but inhibition of ventral MNs, two ganglia anterior to the segment in which it receives a dorsal sensory input. It appears likely that shortening, like local bending and swimming, is produced not by a single IN dedicated to the task, but by a network of INs, some of which do not have input-output functions very similar to the overall behavior.

Supported by NIMH research grant MH43396 and PHS training grant GM07198.

447.9

CHEMICAL DEAFFERENTATION OF INSECTS BY THE ALPHA ADRENERGIC ANTAGONIST PHENTOLAMINE.

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A common approach for elucidating the function of sensory feedback in the generation of rhythmic motor activity is to remove afferent input and analyze the subsequent changes in the motor pattern. Usually deafferentation is done by transection of afferent fibers. However, this method is often technically complicated and usually cannot be done while maintaining records from single neurons. Another approach is to use drugs to inhibit the activity of sense organs. We found that the α_1-adrenergic antagonist Phentolamine inactivates sensory organs in insects when injected into the haemolymph. For the flight behaviour of the locust it can be demonstrated that Phentolamine treatment results in a motor pattern very similar to the deafferented motor pattern as observed after mechanical deafferentation. The inactivation of sense organs is not due to an anaesthetizing action of Phentolamine on the peripheral sensory nerves, since spikes are conducted if electrically evoked. Phentolamine used at concentrations just sufficient to inactivate sensory organs does not abolish efferent activity and muscle activation and has very little effect on the CNS. However, high concentrations of Phentolamine blocked nerve conduction. The inactivation of sense organs without affecting the CNS was specific for α_1-adrenergic antagonists, such as Phentolamine and Tolazoline. α_2-adrenergic antagonists (e.g. Yohimbine), β-adrenergic antagonists (e.g. Propranolol), 5-HT antagonists (e.g. Verapamil) or local anaesthetics (e.g. Lidocaine) could also inactivate sense organs, but only at concentrations which also had a strong central effect. (Supported by the MRC of Canada).

447.10

Timing in acoustical motor following. E.Pöppel, T.Radil.Institute of Medical Psychology LMU,8000 Munich,FRG and Institute of Physiology CSAS,1400 Prague,CSSR

Rhythmic acoustical sequences have been generated by means of a computer and the ability of subjects to follow them by finger tapping analyzed statistically.Usually tapping onset preceded stimulus onset by about 30ms (corresponding to one cycle of the hypothetical brain clock).The duration of this anticipation interval decreased to values close to zero when the pause between the preceding fingercontact with the response key and the following stimulus onset became short (fast tapping,prolongation of stimulus tones combined with an instruction to hold the key) and after training, and increased to values close to about 60ms and more (possible multiples of the basic timing cycle) when the task became more difficult (stimulus sequences with missing tones,false feedback on tapping).Tonal features of stimulus sequences influenced tapping regularity and key touching time was shorter in subjects with musical training.The results are interpreted in the frame of a theory on hierarchical mechanisms of timing in the human brain.

DIFFERENTIATION, MORPHOGENESIS AND DEVELOPMENT: CELLULAR AND MOLECULAR STUDIES III

448.1

FREEZING LESIONS OF THE NEWBORN RAT BRAIN: A MODEL FOR CEREBROCORTICAL MICRODYSGENESIS. P. Humphreys*, G.D. Rosen, G.F. Sherman, and A.M.Galaburda. Beth Israel Hospital and Harvard Med. School, Boston, MA 02215.

Freezing lesions of the neocortex of newborn rats have been suggested as a model of cerebrocortical microdysgenesis, specifically microgyria and *status verrucosus deformis* (Dvorák and Feit, *Acta Neuropath.*, **38**: 203-212, 1977; Dvorák et al., *Acta Neuropath.*, **44**: 121-129, 1978). Using an apparatus similar to that employed by Dvorák and colleagues, we applied freezing probes to the skulls of one day-old rats for periods ranging between 2-10 seconds and with temperatures ranging from -70°C to -110°C. The animals were sacrificed at either weaning or adulthood and their brains examined.

The application of a freezing probe to the skull resulted in substantial focal neuronal loss in the underlying cerebral cortex, with thinning and infolding of the cortex to form a pseudo-sulcus, and accompanying disruption of cortical lamination, thereby replicating the results of Dvorák and colleagues. Longer exposure times and lower temperatures resulted in more severe lesions. In the depth of the pseudo-sulcus, four distinct laminæ were seen which could be the result of (1) laminar necrosis and associated mechanical distortion of growth or (2) disturbed neuronal migration to the supragranular layers. There was no evidence of gliosis within the lesions, although aberrant tufts of myelin (reminiscent of *plaques fibromyeliniques*) were seen at the base of the pseudo-sulci. On the periphery of many lesions were zones of apparent verrucous deformity in layer I, but these resembled neither classic *status verrucosus* nor the ectopic collections of neurons seen in human dyslexic and autoimmune mice (Galaburda et al., *Ann. Neurol.*, **18**: 222-233, 1985;Sherman et al., *Acta Neuropath.*, **74**:239-242, 1987).

These results suggest that early focal cortical injury may mimic neuronal migration abnormalities and lead to significant focal cortical dysplasia.

This work was supported, in part, by NIH Grant 20806, and by the Orton Dyslexia Society.

448.2

CONNECTIONAL ANOMALIES ASSOCIATED WITH FREEZING LESIONS TO THE NEOCORTEX OF THE NEWBORN RAT. G.D. Rosen, P. Humphreys*, G.F. Sherman, and A.M.Galaburda. Beth Israel Hospital and Harvard Med. School, Boston, MA 02215.

Freezing lesions to the left somatosensory neocortex of newborn rats were performed for periods ranging from 2-10 seconds and with temperatures ranging from -70°C to -110°C. At 60 days of age the animals underwent corpus callosectomy. After one week, the animals were sacrificed, their brains removed, sectioned coronally, and processed for silver staining of degenerating axon terminals.

These lesions resulted in substantial focal neuronal loss and laminar dysplasia in the underlying cerebral cortex, and the pattern of transcallosal axonal terminations was disturbed in the surrounding regions. Thus, although transcallosal axonal terminations are rare in somatosensory cortex, there was a pattern of dense terminal degeneration in portions of somatosensory cortex adjacent to the lesions produced by freezing. This is in direct contrast to a paucity of axonal terminal degeneration in the homologous area of the opposite hemisphere, and in analogous regions of control brains. This pattern of callosal axonal degeneration is different from that previously reported in an area of spontaneous cerebrocortical microdysgenesis in the rat and may reflect differences in the etiology (cause or timing) of the cortical disruptions.

We have demonstrated that freezing lesions of the neocortex performed in the newborn rat during the later stages of neuronal migration lead to an alteration in callosal connections in the surrounding regions.

This work was supported, in part, by NIH Grant 20806, and by the Orton Dyslexia Society.

448.3

COMPARATIVE NEUROGENESIS OF PEPTIDE CONTAINING CELLS IN NEOCORTEX OF RAT. M.E. Cavanagh* and J.G. Parnavelas* (SPON: Brain Research Association) Dept. of Anatomy, University College London, London WC1E 6BT, U.K.

Immunocytochemistry, to identify somatostatin (SRIF) Neuropeptide Y (NPY) and vasoactive intestinal polypeptide (VIP) containing neurons, was combined with [3H]thymidine autoradiography (ARG) to determine birthdates and follow the maturation of these cells in rat neocortex. Timemated pregnant rats were injected with [3H]thymidine on days E14-E22. Pups were fixed from birth to 5 weeks of age. PAP immunocytochemistry was performed on 10 µm sections cut from wax-embedded brains, followed by ARG. The numbers, positions and degree of ARG-labelling of peptide-positive neurons were recorded. At two weeks, the peak generation of neurons was from E16 for SRIF, E17 for NPY and E19 for VIP. Neurons generated either side of the peak were usually to be found in the appropriate cortical layer whereas neurons from the peak were rather widely distributed giving the impression that they were not obeying the "inside-out" rule. The apparent numbers of all three types increased initially up to about 3 weeks, then declined. SRIF-neurons appeared first followed by NPY then VIP. The distinct peaks of generation of these neurons may indicate that they arise from a single precursor population which produces them successively.

448.4

DIFFERENTIATION OF PYRAMIDAL AND GABAERGIC NONPYRAMIDAL CELLS IN SLICE CULTURES OF THE HIPPOCAMPUS: GOLGI/EM AND IMMUNOCYTOCHEMICAL STUDIES. M.Frotscher,B.Heimrich* and H.Schwegler*. Inst. Anat.,Univ.Frankfurt/M., FRG.

Organotypic slice cultures offer the possibility of studying the development of neurons in the absence of their specific extrinsic afferents. The combined Golgi/electron microscope (EM) technique and glutamate decarboxylase (GAD) immunocytochemistry were used to study the differentiation of pyramidal and GABAergic nonpyramidal neurons in slice cultures taken from 4-6 day old rats and mice.

Golgi-impregnated cultures revealed all major cell types of the hippocampus. Abundant synaptic contacts on gold-toned pyramidal cell dendrites and spines, normally occupied by extrinsic afferents, suggest sprouting of the cultured neurons. In fact, intrinsic mossy fibers and terminals of intrinsic GAD-positive neurons formed numerous synaptic contacts.

Our results demonstrate that hippocampal neurons develop their cell-specific characteristics, including characteristic synapses, in the absence of extrinsic afferents.

448.5

EARLY DEVELOPMENT OF VAGAL SENSORY AND MOTOR FIBERS AND THEIR NUCLEI IN THE BRAIN STEM OF THE RAT. M. Kalia. Dept. Pharmacology, Thomas Jefferson Univ. Philadelphia, PA 19107.

This study examines the complex interactions between developing vagal afferents and efferents and their neurochemically identified central targets in the brain stem in order to better define the role of structural and neurochemical abnormalities in the brain stems of SIDS victims in the pathogenesis of that disease. Neonatal Sprague Dawley rats from 0-14, 21 and 39 days old were used. Three types of experiments were performed on littermates: 1) tracing vagal projections; 2) immunocytochemistry with antisera directed against catecholamine synthesizing enzymes, 5HT and selected peptides; 3) ultrastructural examination of vagal nuclei with and without labeling #1 and #2 above. 1) The right vagus nerve was injected with 3-5 ul of choleratoxin conjugated to horseradish peroxidase under hypothermia. Animals survived for 24 hours in a incubator following which the brains were processed using TMB as the chromogen according to the method of Kalia and Mesulam, '80. 2) 10 littermates were perfused at each age for immunocytochemistry (Kalia et al, '85). In view of the small size of the brain stem, serial 50 um sections of each brain stem were incubated in only one antiserum so that important rostrocaudal levels were not missed. 3) Brain stems of littermates perfused for electronmicroscopy were sectioned, stained, osmicated and embedded in plastic for ultrastructural examination. At day 3 vagal projections showed the most proliferation but terminals and neurons in the medulla were only weakly immunoreactive. By day 10 the density of vagal projections was greatly reduced but still not as restricted as in the adult. Immunoreactive neurons and terminals showed a greater dispersion and lagged behind developing vagal fibers during the period of early development. Supported by a grant from the SIDS Alliance.

448.6

TYROSINE HYDROXYLASE (TH) AND OLFACTORY MARKER PROTEIN (OMP) EXPRESSION IN RAT OLFACTORY BULB OF EMBRYOS AND IN CULTURE. Harriet Baker[1] And Albert I. Farbman[2]. [1]Cornell Univ. Med. Coll., White Plains, NY 10605 and [2]Northwestern Univ., Evanston, IL 60208.

TH expression in adult olfactory bulbs is regulated by peripheral afferent neuronal input, that is, either chemical or surgical deafferentation results in the loss of TH activity, mRNA and immunoreactivity (ir). The present studies investigated in the rat, both in vivo and in vitro, the existence of a similar relationship between peripheral afferent innervation and TH expression during development. Afferent innervation of the olfactory bulb in vivo, assessed by OMP-ir, preceded expression of TH-ir by about 5 days (Embryonic day [E]-14 vs E-19, respectively). However, TH-ir neurons, when present, were always found in association with OMP-ir fibers and primarily in a juxtaglomerular position. The lag in TH expression in bulbar neurons may be a consequence of delayed differentiation of the phenotype. En bloc organ cultures of E-15 olfactory bulb and epithelium exhibited extensive expression of both OMP and TH after 7 days in vitro. Cultures of olfactory bulb alone contained only a few lightly stained TH-ir cells. These data suggest that during both normal embryogenesis and in culture the presence of receptor afferent fibers is necessary for initiation of TH expression. Supported by Grant #'s NS23103 and NS06181.

448.7

ABNORMALITIES OF ENTERIC NEURONAL DEVELOPMENT IN TRANSGENIC MICE OVEREXPRESSING THE HOMEOBOX-CONTAINING GENE HOX-1.4. V.M. Tennyson, D. L. Sherman*, R.R. Behringer*, R. L. Brinster*, R. D. Palmiter*, J.Tasch*, D. Crotty*, D.J. Wolgemuth* and M.D. Gershon, Depts. Anat. & Cell Biology, and Genetics & Devel., Columbia Univ., P & S, New York, NY 10032; Lab. of Repro. Physiol., Univ. Penn. School of Vet. Med., Phila, PA, and Dept. Biochem., Univ. Wash., Seattle, WA.

Transgenic mice carrying multiple copies of the Hox-1.4 gene develop congenital megacolon and express elevated levels of Hox-1.4 mRNA in the gut. Megacolon also develops in *ls/ls* mutant mice because crest-derived neural and glial precursors fail to colonize the terminal bowel. The terminal bowel of adult transgenic mice was found to be hypoganglionic and not aganglionic, like that of *ls/ls* mice. The ganglia and nerves in the hypoganglionic zone of the transgenic gut had the ultrastructure of extraenteric peripheral nerve, rather than that of the enteric nervous system (ENS). Scattered neurons were present within thick nerve bundles. In contrast to normal enteric ganglia, these neurons, as well as the nerves in which they were found had a collagen-containing endoneurium; supporting cells were surrounded by basal laminae, often myelinated axons, and thus are Schwann and not enteric glial cells. An abnormal accumulation of material with the ultrastructure of elastic fibers was present. The smooth muscle layers of the most terminal region were unusually thickened and interrupted by fibrous septae. Immature neurons first appeared at E14, as in controls, but sometimes expressed an abnormal phenotype, such as that of sympathetic neurons with 50 nm dense cored granules. The development of the internal smooth muscle layer in the terminal gut of transgenic fetuses was markedly retarded. These data suggest that congenital megacolon develops in the transgenic mouse because the terminal bowel contains small numbers of abnormal neurons, which fail to mediate the local reflexes upon which intestinal propulsion depends. The condition is not identical to that which appears in *ls/ls* mice, although abnormalities of the developing smooth muscle and extracellular matrix are seen in both defects. Supported by NIH grants HD 17736, NS 15547, HD 18122.

448.8

EVIDENCE THAT MOVEMENT IN *DROSOPHILA* EMBRYOS IS NEURALLY EVOKED AND MEDIATED BY GLUTAMATE. M.D.S. Anderson and H. Keshishian. Dept. of Biol. Yale University, New Haven, CT 06511.

It is known that the initial neuromuscular contacts on the bodywall muscles of *Drosophila* occur during stage 16 of embryogenesis and at this time the growth cones of these axons show positive immunostaining for glutamate (Johansen et al., Soc. Neurosci. Abst. 14:451, 1988). This is also the stage when spontaneous muscle movement begins in the embryo. Due to the diffuculty of recording muscle potentials in embryos at this stage, it has not been possible to demonstrate the source of these muscle contractions. We have begun using a glutamate-blocking spider venom fraction to examine the basis of these early muscle contractions.

We have HPLC purified a component of the venom from the Orb Weaver spider *Araneus gemma* that postsynaptically blocks the glutamate response at the neuromuscular junction of *Drosophila* larval bodywall muscles. Glutamate-blocking activities of venom components were tested by physiological means using iontophoresis of glutamate in the presence of various HPLC fractions. We found several with robust postsynaptic effects as open channel blockers and have used one fraction to examine the development of neuromuscular contacts on the bodywall muscle fibers. By microinjecting the glutamate-blocking venom fraction into living embryos, we have been able to reversibly eliminate the muscle movements. Our results suggest that the embryonic muscle contractions are neurally evoked and likely due to glutamate release from the motoneurons. We are also examining similarities between our active fractions and other argiotoxins described in the literature (Adams et al., Bio. Biophys. Res. Comm.148:678 '87).

448.9

PRE-METAMORPHIC DEVELOPMENT OF SEROTONIN IMMUNOREACTIVITY IN *APLYSIA*. R. Marois and T.J. Carew. Departments of Psychology and Biology, Yale University, New Haven, CT 06520

Serotonin (5-HT) has been implicated in a variety of forms of behavioral and neuronal modulation in *Aplysia*. We have begun to explore complementary roles that 5-HT neurons may have in neurogenesis by tracing the expression of serotonin immunoreactivity (SIR) in embryonic and larval *Aplysia*.

The first SIR cells appear in the cerebral ganglia (CG) after about 50% of embryonic development. They consist of one bilaterally symmetrical pair of cells and one unpaired median cell. These cells send an anterior projection that follows the outline of the velar lobes and a medial projection to a SIR neuritic plexus in the CG. Some SIR fibers originating from the plexus project ventrally to the foot primordium. At hatching, a new pair of bilaterally symmetrical SIR cells appears in the CG and projects into the 5-HT plexus. Also at this time a caudal loop of SIR fibers extends from the CG into the visceral mass.

After hatching, the pattern of SIR undergoes little change until Stage 3 of larval development when 2-3 cells appear in each pedal ganglion (PeG). Some ventral projections from each PeG converge medially and innervate the developing foot and propodium, while others innervate the lateral aspects of the foot. Soon thereafter, SIR fibers cross the dorsal pedal commissure. By Stage 4 a single SIR cell appears along the visceral SIR loop, which is now twisted by about 90^{0}. This basic SIR scaffolding remains intact until animals acquire competence to metamorphose at Stage 6. By this time 2 more bilateral pairs of CG cells have been added and each PeG contains about 6-8 cells.

We are currently examining: (1) the impact of metamorphosis on the larval SIR pattern, and (2) the relationship of these SIR cells to identified 5-HT neurons of juvenile and adult *Aplysia*. We will then be in a position to address the hypothesis that identified 5-HT modulatory neurons of adult *Aplysia* might play organizational roles in the development of the CNS.

448.10

REGULATION OF NEUROPEPTIDE PHENOTYPES IN IDENTIFIED NEURONS OF MANDUCA EMBRYOS. J.B. Wall* and P.H. Taghert. Department of Anatomy & Neurobiology, Washington University School of Medicine, St. Louis, MO 63110

We are interested in the developmental regulation of transmitter phenotypes. We have studied two types of FMRFamide-immunoreactive (F-IR) neuroendocrine cells that project to a common nerve in the moth Manduca: the peripheral L1 neuron and the CF neurons of the CNS. The CF cells and the L1 cells contain distinct populations of immunoreactive secretory granules. Polyclonal antisera to four different epitopes of the Drosophila DPKQDFMRFamide gene stain the CF neurons specifically, but do not stain the L1 neuron. This suggests that the CF cells express the Manduca homologue of the Drosophila gene, while the L1 cells express a different gene encoding a separate set of FMRFamide-like peptides. Embryonic differentiation of the L1 and CF cells was monitored using intracellular dye fills and anti-FMRFamide antisera. The L1 neurons becomes F-IR at 42% of development, just after axonogenesis begins (axon ~35 um). In contrast, CF neurons become F-IR at 60%, only after extending a long (~300 um) axon from the CNS to the periphery. In both cases, the onset of neuropeptide expression correlates well with extensive contacts made by the respective neuronal growth cones onto an identified, mesodermally-derived syncytial cell that lies near the developing nerve. Identified motorneuron growth cones growing in the same vicinity do not display this affinity for the syncytial cell. L1 and CF axonal affinity for the syncytial cell is transient and typically not seen after ~80% of development. These observations suggest that cell type-specific initiation of neuropeptide expression seen in the L1 and CF peptidergic cell types may be influenced by cellular interactions with a common intermediate target. Electron microscopy, embryo culture and specific cell ablations are being used to investigate this phenomenon. Supported by NIH grant #NS21749 to PHT.

449.1

EFFECTS OF APORPHINES ON REGIONAL BRAIN NEUROTENSIN CONCENTRATIONS: IMPLICATIONS FOR DEVELOPMENT OF NOVEL ANTIPSYCHOTIC DRUGS. C.B. Nemeroff, C.D. Kilts,* B. Levant, A. Campbell,* G. Bissette, J.L. Neumeyer and R.J. Baldessarini. Depts. Psychiat. & Pharmacol., Duke Univ. Med. Ctr., Durham, NC 27710 and McLean Hosp., Harvard Univ., Belmont, MA 02178.

Considerable data have revealed interactions between neurotensin (NT) and dopamine (DA) neurons. Centrally administered NT possesses many properties in common with clinically efficacious antipsychotic drugs. We have suggested that antipsychotic-induced increases of NT in the n. accumbens (NA) are correlated with antipsychotic efficacy whereas NT increases in the caudate are associated with extrapyramidal side effect (EPS) liability. We sought to determine whether chronic treatment with S(+) NPA, an isomer of N-n-propylnorapomorphine (NPA) with selective inhibiting effects on mesolimbic DA systems alters regional brain NT concentrations. Adult male S-D rats were treated chronically with haloperidol (3 mg/kg), S(+) NPA (3 mg/kg tid), R(-) NPA or vehicle for 10-15 days. NT was measured in 11 brain regions. Both haloperidol and S(+) NPA, but not R(-) NPA, produced significant increases in NT concentrations in the NA. In the caudate nucleus, haloperidol increased NT concentrations but S(+) NPA and R(-) NPA did not. These data provide further evidence for increases in NA NT concentrations by putative antipsychotic drugs. (Supported by NIMH MH-39415, MH-39967, MH-34006 and MH-47370)

449.2

CSF NEUROTENSIN CONCENTRATIONS IN PSYCHOSES. G. Bissette, D. Garver, K. Kelly* and C.B. Nemeroff. Depts. Psychiatry and Pharmacology, Duke Univ. Med. Ctr., Durham, NC and Univ. Cincinnati, Cincinnati, OH.

Neurotensin (NT) is an endogenous neuropeptide transmitter that shares many of the pharmacobehavioral effects of antipsychotic drugs in laboratory animals. The CSF concentration of NT has been previously shown to be decreased in drug-free schizophrenic patients compared to controls. In the present study, the CSF concentration of NT of 6 normal controls and 20 psychotic (DSM-IIIR diagnosis of schizophrenia [N=15], schizophreniform disorder [N=2] or psychotic mood-incongruent affective disorder [N=3]) patients were compared after a drug-free period of two weeks. In the psychotic patients (N=8) with CSF NT concentrations below the 40th percentile, significantly greater "formal thought disorder", "behavioral disorganization", "impaired functioning" and "incidence of delusions and hallucinations" was observed in the drug-free state. Seven of eight of the patients below the 40th percentile in CSF NT concentrations were also delayed in their therapeutic response to haloperidol after failing to respond to lithium treatment. This data suggests that a subpopulation of psychotic patients with lowered concentrations of NT in CSF have more severe psychotic symptoms and exhibit a delayed response to antipsychotic drug treatment. Supported by NIMH MH-39415 and Scottish Rite Schizophrenia Research Foundation.

449.3

SPECIFICITY OF ACTION OF CCK-TETRAPEPTIDE IN PANIC DISORDER. J. BRADWEJN, D. KOSZYCKI*, C.SHRIQUI*, G. METERISSIAN*. Division of Psychopharmacology, St.Mary's Hospital, McGill University, Montreal, Quebec, H3T 1M5

Cholecystokinin (CCK) is a peptide, fulfilling criteria for a neurotransmitter, found in high concentrations in the mammalian CNS. CCK-tetrapeptide (CCK-4) has been reported to induce panic attacks in panic-disordered patients (PD) (Bradwejn J et al, Neuroscience 1988) and in healthy volunteers (HV) (de Montigny C, Neuroscience 1988). The purpose of this study was to determine the specificity of action of 50 ug of CCK-4 in panic disorder. 50 ug of CCK-4 were injected IV to 11 PD (5F, 6M), mean age (± SEM): 33.5 ± 3.6 years; and 15 HV (5F, 10M), mean age: 27.3 ± 7.1 years. A double-blind, randomized sequence of injections of CCK-4 and placebo (NaCl 0.9%) was used. Incidence, time of onset, number of symptoms and duration of CCK-4-induced panic attacks for PD and HV respectively were as follows: (mean ± SEM) 100% and 40%, 20 ± 3 and 21 ± 2 secs, 12.7 ± .66 and 14.3 ± .88, 20.7 ± 4.3 and 4.3 ± 2.7 mins. The significant difference in incidence ($P<.006$) and duration ($P<.003$) for PD and HV suggest a threshold specificity for panicogenic effect of CCK-4 in PD.

449.4

FENFLURAMINE STIMULATION OF HORMONE RELEASE IN OBSESSIVE COMPULSIVE DISORDER. W.A. Hewlett*,W.S. Agras and S. Berman Dept. of Psychiatry, Stanford University School of Medicine Stanford.CA. 94305.

Obsessive-Compulsive Disorder is a Psychiatric Disorder characterized by the presence of unwanted thoughts and stereotyped behaviors performed to reduce the anxiety associated with those thoughts. Serotonergic agents are known to affect the symptoms of this disorder and serotonergic hypotheses related to this disorder have been proposed. To study the relationship between CNS serotonergic state and the presence of OCD, 21 patients and 20 normal controls were administered a 60mg oral dose of fenfluramine, a substituted amphetamine which promotes the release of neuronal serotonin. Plasma hormone levels were measured in 2 baseline samples drawn prior to fenfluramine administration and at hourly intervals for 5 hours post-administration.Results indicate that fenfluramine produces a significant elevation in plasma prolactin levels in both patients (176%) and controls (285%). Female subjects have a significantly increased prolactin responce to fenfluramine stimulation when compared to male subjects($p<.0001$). Prolactin responses are blunted in Obsessive-Compulsive patients relative to controls. This effect is attributable to a blunting of the prolactin response in obsessive emales($p<.05$) since no differences are observed between obsessive males and their controls.

449.5

ALTERATIONS IN BILATERAL DISTRIBUTION OF BETA-RECEPTOR SUBTYPES IN SCHIZOPHRENICS AND SUICIDES

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We have employed quantitative autoradiography to examine the distribution of $Beta_1$ and $Beta_2$ receptors in both hemispheres in several regions of postmortem brain tissue from: (i) schizophrenic patients that did not commit suicide, (ii) schizophrenic patients that did commit suicide, (iii) nonschizophrenic suicide cases and (iv) nonpsychotic, nonsuicide controls. In hippocampus, thalamus and striatum an asymmetric distribution of Beta receptors was evident in the controls. The $Beta_2$ subtype was higher in density in the right hemisphere of the hippocampus and thalamus, $Beta_1$ was higher in the right striatum. In schizophrenia this asymmetry was altered with an increase in $Beta_2$ receptor density in the left hippocampus and a decrease in the right hippocampus. Similar results for $Beta_1$ receptor density were observed in limbic cotrical but not other cortical regions. In a number of nuclei of the amygdala there was a decrease in the left hemisphere in total Beta density. Patients who committed suicide with or without a diagnosis of schizophrenia showed increased $Beta_1$ receptor density in the amygdala of the right hemisphere and decreased density in the thalamus. In contrast, schizophrenics who committed suicide showed different effects in hippocampus when compared to suicides without psychosis. Suicides showed consistent decreases in $Beta_2$ receptor density in both hemispheres, while shizophrenics committing suicide showed an increase in the left hemisphere (like nonsuicide schizophrenics). **Funded by NIH NS19597, MH 43852 and MH43880.**

449.6

DEFICITS IN SMALL INTERNEURONS IN SCHIZOPHRENIC CORTEX. F.M. Benes, J. McSparren*, J.P. Sangiovanni*, S.L. Vincent. Dept. of Psychiatry, Harvard Medical School and Mailman Research Center, McLean Hospital, Belmont, MA.

In order to replicate and extend an earlier finding of lower neuronal density in cerebral cortex of schizophrenics, a second patient cohort containing both chronic undifferentiated (N = 9) and schizoaffective (N = 9) subtypes of schizophrenia has been obtained. Nissl-stained sections of prefrontal and anterior cingulate cortex have been codified and subjected to a blind, random sampling paradigm. The results indicate that both types of schizophrenic patient showed lower densities of small interneurons in layers II through VI when compared to normal controls (N = 12). Two young patients (aged 23 and 32 yrs) with no neuroleptic exposure also showed lower cell counts. Since the laminar distributions of these findings overlaps that of basket, chandelier and neuroglia-form cells, we postulate that our data may possibly reflect a deficit of inhibitory GABAergic interneurons in chronically psychotic patients. It is not possible as yet to determine whether these changes arose before, during or after the onset of schizophrenia, although our current evidence shows that these differences are present early in the course of the illness and cannot be explained by various confounding effects. Supported by MH00423, MH42261, MH31154, MH/NS31862 and the Scottish Rite Foundation.

449.7

CYTOARCHITECTURAL ABNORMALITIES OF THE ENTORHINAL CORTEX IN SCHIZOPHRENIA. S.E. Arnold*, B.T. Hyman and G.W. Van Hoesen (SPON: S.A. Moore). Depts. of Neurology and Anatomy, Univ. of Iowa, Iowa City, IA 52242.

The cytoarchitecture of the entorhinal cortex was examined in the brains of 6 patients with a diagnosis of schizophrenia and in 13 controls. All 6 schizophrenic brains showed cytoarchitectural abnormalities of the rostral and intermediate portions of the entorhinal cortex. The abnormalities included aberrant non-sulcal invaginations of the anterior parahippocampal gyrus with disruption of cortical layers, heterotopic displacement of laminar-specific neurons and paucity of neurons in superficial layers. Such changes would be consistent with disturbed development. The entorhinal cortex is a pivotal point for neural systems that mediate cortico-hippocampal and hippocampo-cortical interactions. The disruption of these neural systems may have important neuropsychological sequelae, and contribute to the symptoms of schizophrenia. (Supported by: NS 14944.)

449.8

THE ROLE OF THE RIGHT BASOTEMPORAL CORTEX IN THE PRODUCTION OF SECONDARY MANIA H.S.Mayberg, S.E.Starkstein, R.G.Robinson, H.Wagner Department of Psychiatry, Johns Hopkins University School of Medicine, Baltimore, MD 21205

Secondary mania (SM) (i.e., mania after a brain injury) has been reported in association with lesions involving cortical (basotemporal and orbitofrontal) or subcortical (caudate or thalamus) regions of the right hemisphere (Starkstein et al., Arch Neurol 44:1069, 1987). We present a new series of 8 patients with SM having lesions involving cortical (right basotemporal cortex in 4 cases, and bilateral orbitofrontal cortex in 1 case), and subcortical (right head of the caudate in 2 cases and right frontal white matter in 1 case) brain regions. Positron Emission Tomography (PET) studies using 18-Fluorodeoxyglucose (^{18}FDG) were performed in 3 patients with subcortical lesions and SM, in two patients with subcortical lesions without mania, and in 3 normal, age-comparable controls. The ^{18}FDG-PET scan studies demonstrated right basotemporal cortex hypometabolism in all three manic patients, but in none of the lesion or normal controls ($F(2,5)=6.32, p<.05$). This finding suggests that the mechanisms leading to SM may involve the right basotemporal cortex. Dysfunction of the basotemporal cortex may result from remote hypometabolic effects (diaschisis) or from lesions directly involving the basotemporal cortex.

449.9

BIPOLAR AFFECTIVE VERSUS PURE MANIC DISORDER AFTER BRAIN LESIONS R.G.Robinson, S.E.Starkstein, P.Fedoroff*, M.L. Berthier*, H.S.Mayberg, P.R.McHugh Department of Psychiatry, Johns Hopkins University School of Medicine, Baltimore, MD 21205

Although mania is a rare complication of brain injury, recent studies have found that lesion location, genetic predisposition and subcortical brain atrophy are important correlates of this affective syndrome (Robinson et al., Am J Psychiat 145:172, 1988). In the present study we compared patients who developed a bipolar affective disorder (i.e., mania and depression) after a brain lesion (n=7), with patients that developed only mania (n=12) (i.e., unipolar disorder). Although no significant between-group differences were found in demographic variables, the manic depressed group had significantly greater cognitive impairment than the mania only group (Mini-Mental State Exam Scores [mean±SD]: 25.2±4.7 and 28.6±1.8, respectively, $t=2.21$, $p<.05$). In addition, all of the bipolar patients had subcortical lesions (mainly involving the right head of the caudate and the right thalamus). In contrast, 10 of 12 patients with unipolar mania had cortical involvement (mainly right orbitofrontal and basotemporal cortices) (X^2 Yates=7.38, $p<.01$). These findings suggest that subcortical and cortical right hemisphere lesions may produce different neurochemical and/or remote metabolic brain changes which may underlie the production of either a bipolar disease or a unipolar mania.

449.10

AMPHETAMINE AND CEREBRAL BLOOD FLOW (XE-133 DYNAMIC SPECT) IN SCHIZOPHRENIA. D.G. Daniel*, D.W. Jones*, J.R. Zigun*, R. Coppola, L.B. Bigelow*, K.F. Berman, T.E. Goldberg* and D.R. Weinberger. NIMH Neurosciences Center at St. Elizabeths, Washington, D.C. 20032.

Several lines of evidence suggest a monoaminergic role in the pathophysiology of reduced prefrontal metabolism in schizophrenia. Recently, we observed that apomorphine, a direct dopamine agonist, increased prefrontal cortex (PFC) blood flow (Xe-133 rCBF) during the Wisconsin Card Sort (WCS), a PFC-linked cognitive task. To further explore the role of monoamines on cerebral function we conducted a double-blind placebo controlled cross-over study of the effects of .25mg/kg dextroamphetamine on cerebral blood flow (CBF) as determined by Xe-133 dynamic single photon emission computed tomography (SPECT) during performance of the WCS and a simple control task. Subjects included 9 patients with chronic schizophrenia who had been stabilized for six weeks on .4mg/kg haloperidol.

Amphetamine produced a non-significant trend towards a diffuse reduction in rCBF that was independent of task. On placebo, no significant activation of CBF was seen during the WCS compared with the control task. With amphetamine, however, significant task specific activation of the left dorso-lateral prefrontal cortex occurred (paired $t = 4.75$, $p=.0015$). The results are further evidence that enhanced PFC monoaminergic activity may increase PFC metabolism and reverse "hypofrontality" in schizophrenia. In addition, on amphetamine, but not on placebo, a significant correlation ($r=.73$, $p=.04$) emerged between activation of PFC CBF and performance of the WCS task. These findings are consistent with animal models in which mesocortical dopamine activity modulates and enhances the signal to noise ratio of PFC activity.

449.11

VALIDATION OF PET D_2 DOPAMINE RECEPTOR QUANTIFICATION USING [C-11] NMSP AND [F-18] HALOPERIDOL D.F. Wong*, D. Young*, L.T. Young*, L.E. Tune*, G. Pearlson, E. Minkin*, C. Cidis Meltzer*, K. Midha*, R.F. Dannals*, H.T. Ravert*, A.A. Wilson*, E.D. London, H.N. Wagner,Jr.*, M. Casanova* J. Klineman*, M.J. Kuhar, A. Gjedde* (SPON: E.Broussolle). Johns Hopkins Med. Inst., U. of MD, Addiction Research Center NIDA, Baltimore. MD, U Sask., NIMH Washington, Montreal Neuro Inst.

We reported elevated D_2 dopamine receptor B_{max} in drug naive schizophrenics (SCZ) (Science 234:1558, 1986), as shown by PET with [C-11] 3-N-methylspiperone (NMSP). We have tested whether the following methodological issues contributed to our findings: [C-11] labelled metabolite corrections, plasma protein binding, and brain HAL partition coefficients. We found: (1) Multiple regression with a fitted curve showed no significant differences between plasma [C-11] NMSP metabolites measured by HPLC and modelled input function corrections (N=36). (2) There were no differences in plasma protein binding of either [C-11] NMSP in 21 patients, 8 SCZ (95.7 ± 0.4%),11 controls (95.6 ± 0.4%), and 3 bipolars (93.4 ± 2.0%); or [F-18] HAL in 10 SCZ (97.3 ± 0.2%), 6 normals (98.3 ± 0.2%), and 6 bipolars (97.2 ± 0.2%). (3) Biodistribution studies of [F-18] HAL in mouse brain performed in the presence of D_2 and *sigma* receptor antagonists gave results consistent with previous [H-3] HAL studies. No differences were found in [F-18] HAL partition coefficients in postmortem brain from 7 normals (3.1 ± 0.8 g tissue/ml plasma), 6 SCZ (3.1 ± 1.2), or 6 bipolars (2.9 ± 0.6), and values correlated closely with model assumptions. We continue to find elevated mean D_2 B_{max} with these methodologic improvements in 19 drug naive SCZ (34.2 ± 19 pmol/g) and 7 psychotic bipolars (28.7 ± 12) compared with 19 controls (15.1 ± 8.6) and 7 non-psychotic bipolars (16.9 ± 8).

449.12

VISUAL PATTERN-COMPLETION IN NORMAL AND SCHIZOPHRENIC SUBJECTS. B. Ott*, O.-J. Grüsser, Katharina Berndl* (SPON: ENA). Dept. of Physiol., Freie Universität Berlin, Arnimallee 22, 1000 Berlin 33 (West), Germany

In 37 schizophrenic subjects and a group of normal controls (age, gender and socially matched to subjects) visual object recognition was investigated in a slide projection test (b/w photographs and line drawings). Data were obtained from 9 different series of 12 slides each. The objects were presented as: (a) complete object; (b) partially visible object; (c) partially visible objects in context with other objects; (d) objects masked by meaningless patterns. (e) In another series a characteristic object in a simple scene was missing and had to be named. (f) A part lacking in a single object had to be named. (g) An event preceding a certain situation had to be named.

Total error score from all series was 12.4 + 1.0 (s.e.) percent for normals and 27.4 +2.0 percent for patients. No significant disparity between hebephrenic and paranoic adult schizophrenics was found. The average error score as well as the error scores in the 9 subtests differed for normals and schizophrenics at a significance level of $p<0.001$.

The data only slightly depended on the age of patients and normal subjects (linear correlation coefficient r=0.35 or 0.50 respectively), while level of education, duration of disease or hospitalization, and schizophrenic defect had an insignificant effect, if at all.

The data are interpreted as an indication of a *general cognitive deficiency* in schizophrenia, appearing in the present study for the visual domain. (Supported in part by a DFG-grant, Gr 161).

450.1

CHARACTERIZATION OF PROENKEPHALIN GENE EXPRESSION IN DEVELOPING FROG. M. Wong*, R. Rius*, W. P. Hayes, and Y.P. Loh. (SPON: R.Thompson). Laboratory of Developmental Neurobiology, NICHD, Bethesda, MD 20892.

In order to characterize factors which regulate the spatial and temporal expression of the opiate neuropeptide, met-enkephalin, the proenkephalin gene has been cloned in Xenopus laevis. A 2 kb probe consisting of the third exon of the frog proenkephalin gene (obtained from G. Martens; Martens, G., *Nature* 310:251, 1984) was used to screen a Xenopus laevis genomic library (obtained from I. Dawid, NICHD). Restriction enzyme map analysis showed that two positive clones in size 12 and 16 kb were isolated. These were shown to contain about 6 and 8 kb of 5' DNA sequences. Sequencing indicated that both clones were coding for the same gene. The 5' upstream flanking region of the 8 kb clone is currently being analyzed to identify the TATA box and other putative regulatory sequences.

In situ hybridization studies to localize the onset of brain and pituitary proenkephalin expression during embryogenesis are also in progress. The distribution of proenkephalin cells has been mapped in adult brain, and a similar pattern of expression was found to be present in tadpoles at developmental day 4.5 (Stage 46). In addition, cells expressing proenkephalin mRNA were found in the rostral part of the anterior pituitary.

Future studies will examine the effect of deleting the specific 5' upstream regulatory elements that have been identified on the initiation and patterning of proenkephalin gene expression.

450.2

GENE EXPRESSION OF A NOVEL MAMMALIAN NEUROENDOCRINE POLYPEPTIDE (7B2) IN LOWER VERTEBRATES AND IN THE MARINE MOLLUSK *APLYSIA CALIFORNICA*. L. Gaspar*, M. Mbikay*, M. Chrétien* and V.F. Castellucci. Clinical Research Institute of Montreal, Montreal, (Que), Canada, H2W 1R7.

A highly conserved polypeptide called 7B2 has been isolated from porcine and human pituitaries. This polypeptide is also widely distributed in the CNS but its biological role is not yet known.

The mouse cDNA sequence has been determined (Mbikay et al., 1989). Northern and Southern blot analysis shows the expression of the 7B2 gene in other mammalian species (cow, rat, monkey) and also in other vertebrates such as chicken and fish. Since the 7B2 gene appears to be highly conserved throughout evolution, we decided to study the presence of this polypeptide in the marine mollusk *Aplysia californica*. By using a probe derived from the mouse 7B2 sequence, Northern blot hybridizations under highly stringent conditions were carried out. They revealed signals in the mRNA population of different organs such as ovotestis, hepatopancreas, stomach, kidney and CNS of *Aplysia*. Similarly, signals were detected by Southern blot analysis in this invertebrate.

These preliminary results suggest that the 7B2 gene might be expressed in this system providing a useful tool for further investigation of the biological role of this polypeptide family.

450.3

ANGIOTENSIN II IS EXPRESSED IN SOLITARY VASOPRESSIN CELLS WITH A HETEROZYGOUS PHENOTYPE IN HOMOZYGOUS BRATTLEBORO RATS. F.W. van Leeuwen*, H. Imboden** and D. Felix**. *Netherlands Institute for Brain Research, Amsterdam, The Netherlands, **University of Berne, Switzerland.

In the homozgyous Brattleboro rat (di/di) an altered vasopressin (VP) precursor is synthetized resulting in reduced hormone packaging. The expression of Angiotensin II (Ang II) immunoreactivity is also disturbed, and although dynorphin (DYN) is present in smaller vesicles than in heterozygous controls, it is not affected in di/di rats.

Paradoxically, a small number of solitary hypothalamic neurons of di/di rats synthetizes the wild-type VP precursor (i.e. VP, C-terminal VP-neurophysin and a glycopeptide). Previously we provided evidence that during life an increasing number of these post-mitotic neurons (up to 3% of the VP cells) undergo a switch to a genuine heterozygous phenotype. Thus, as di/di rats age, these hypothalamic neurons exhibit both the wild-type and the mutated VP precursors. Here we report the presence of Ang II in these heterozygous cells indicating that for the expression of Ang II a normal VP precursor is necessary. It is hypothesized that in the Golgi apparatus the various neuropeptides which are present in VP cells are packaged in granules in the following order: DYN first, followed by VP and Ang II.

450.4

CALCIUM CHANNEL MODULATION AND RESPONSE ELEMENTS OF THE RAT PROLACTIN GENE. **R.N. Day*, B.A. Biagi* R.A. Maurer*, and J.J. Enyeart*. (SPON: A. Rotter). Dept. of Physiol. Biophys., Univ. of Iowa, Iowa City, IA 522242 and Dept. of Pharmacology, Ohio State Univ., Columbus, OH 43210.**

In some endocrine cells, hormone synthesis may be regulated by Ca^{2+} entry through voltage-gated ion channels. We have used dihydropyridine (DHP) Ca^{2+} channel modulators to explore the relationship between Ca^{2+} channels and prolactin (PRL) synthesis in pituitary cells, and to identify specific Ca^{2+} response elements within the 5' flanking region of the rat PRL gene. In patch clamp experiments, enantiomers of the DHP Bay K8644 produced opposing effects on Ca^{2+} current through L-type Ca^{2+} channels in GH_3 and GH_4C_1 pituitary cells. (-)-Bay K8644 enhanced the magnitude of Ca^{2+} currents 2-5 fold, while (+)-Bay K8644 blocked L channels. The enantiomers also produced opposite effects on PRL production by GH and normal rat pituitary cells in culture. Over 72 hours, cells treated with 500 nM (-)-Bay K8644 typically produced 3-4 times more PRL than controls while the (+)isomer inhibited PRL production by 30-50%.

To assess the role of the 5' flanking sequences of the rat PRL gene in conferring Ca^{2+} and DHP regulaton of transcription, several fusion gene constructs containing 5' flanking sequences were coupled to the reporter gene bacterial chloramphenical transferase (CAT) and transfected into GH_3 cells. Ca^{2+} addition stimulated CAT expression in cells containing a construct which included 1.9 kilobase pairs of the 5' flanking sequence and the effect was enhanced by (-)-Bay K8644. Constructs containing either proximal (positions -292 to -38) or distal (positions -1714 to -1495) enhancer elements of the PRL gene also responded to Ca^{2+} and the agonist with enhanced expression of CAT. The additive response observed with the two enhancer elements (9 fold) was equal to that observed with the 1.9 kilobase construct alone. In conclusion, the enantiomers of Bay K8644, by enhancing or blocking Ca^{2+} movement through "L" type channels in pituitary cells, produce opposing effects on PRL synthesis. These responses may be mediated by two separate Ca^{2+} response elements in the proximal and distal enhancer regions of the rat PRL gene.

450.5

REGULATED EXPRESSION OF α- and β-CGRP PROMOTERS IN TRANSFECTED CELL LINES.
M.A.Kirschner* and S.G.Amara, Section of Molecular Neurobiology, Howard Hughes Medical Institute, Yale Univ. School of Medicine, 333 Cedar Street, New Haven CT, 06510.

The calcitonin/α-CGRP and β-CGRP genes code for two, nearly identical neuropeptides that are expressed differentially and in discrete regions in the nervous system. Because they appear to be independently regulated and have highly divergent promoter sequences, they offer an excellent model system in which to explore the determinants of tissue-specific gene expression. We have investigated the regulatory role of 5' upstream sequences of α- and β-CGRP genes by analyzing the expression of gene constructs introduced into cells after transient DNA transfection. Promoter regions have been fused to reporter genes encoding chloramphenicol acetyl transferase (CAT) and β-galactosidase. Constructs containing 2.0 and 2.3 kilobases of α- and β-CGRP 5'-flanking regions, respectively, are sufficient to direct the expression of CAT activity in PC12 cells. Constitutive α- and β-CGRP promoter-driven expression is low in PC12 cells relative to a strong viral promoter (SV40). However, the α-CGRP promoter activity is induced two-fold after exposure to 10 μM forskolin, thus supporting a role for agents which elevate cAMP in the transcriptional regulation of the α-CGRP/calcitonin gene. Deletion mapping of the upstream promoters is currently being utilized to further delineate functional regions that mediate observed hormonal effects.

450.6

FOS AND JUN COOPERATE TO ACTIVATE TRANSCRIPTION OF THE HUMAN VIP GENE. M. Verhave*, R. Goodman* and J.S. Fink. Div. of Molecular Medicine, New England Medical Center Hospitals, Boston, MA 02111 and Lab. of Molecular Neurobiology, Department of Neurology, Massachusetts General Hospital, Boston, MA 02114 (SPON: A. Boukoms).

Transcription of the human VIP gene by cAMP or phorbol esters requires the integrity of an 17 bp enhancer element located near to its promotor. The sequence of the VIP second messenger-responsive element is similar to that of a TPA-responsive element (TRE) which interacts with the transcriptional activator protein AP-1. The AP-1 family of enhancer binding proteins includes the protein products of the *fos* and *jun* proto-oncogenes. Fos and Jun proteins form a heterodimeric complex that interacts with AP-1 binding sites in several TREs. In this study we sought to determine (1) if Jun /AP-1 interacts with the VIP second messenger enhancer, and (2) if Jun and Fos cooperate to activate transcription of the VIP gene. Expression vectors containing *v-jun* or human *c-fos* cDNAs were co-transfected into HeLa cells with fusion genes containing 94 bp of the 5'-flanking region of the VIP gene (containing the second messenger enhancer) linked to the reporter gene chloramphenicol acetlytransferase (CAT). There was little or no transactivation of the VIP-CAT gene by the *v-jun* or *c-fos* expression vectors when transfected alone with VIP-CAT. However, when *v-jun* and *c-fos* expression vectors were transfected together, there was a 10 to 20-fold transactivation of the VIP-CAT gene. Similar results were obtained when the 17 bp VIP enhancer element linked to a viral promotor was used as a target gene. In a DNase 1 footprinting assay purified AP-1, v-Jun or c-Jun protein protected a region of the VIP gene containing the second messenger enhancer. We conlcude that transcriptional activation of the VIP gene by second messengers such as cAMP or activators of protein kinase C may require the interaction of Jun/c-Fos complexes with the VIP second messenger enhancer.

450.7

CYCLIC AMP- AND GLUCOCORTICOID-MEDIATED TRANSCRIPTIONAL REGULATION OF RAT PROENKEPHALIN GENE EXPRESSION IN C6 RAT GLIOMA CELLS. J. Joshi* and S.L. Sabol. Lab. of Biochemical Genetics, NHLBI, NIH, Bethesda, MD 20892.

Glucocorticoids potentiate proenkephalin (pEnk) gene expression in several systems. Yoshikawa and Sabol (*Mol. Brain Res.* 1, 75, 1986) showed that glucocorticoids such as dexamethasone (Dex) and adenylate cyclase activators synergistically elevate the pEnk mRNA abundance in C6 rat glioma cells. We have investigated the mechanism of this effect by nuclear run-on transcription analyses and transfection assays. The pEnk gene transcription rate was not significantly altered by 1 μM Dex alone, transiently stimulated by 20 μM forskolin (up to 6-fold at 1 hr), and more persistently stimulated by Dex + forskolin (3-6-fold over 1-24 hr). Cycloheximide did not block these increases, indicating no requirement for ongoing protein synthesis. Increases in pEnk mRNA levels in C6 cells paralleled the transcription rate increases. Dex did not alter the basal or forskolin-stimulated cAMP content of the cells and produced only a modest (30%) cycloheximide-sensitive elevation of cAMP-dependent protein kinase activity. These results suggest that cAMP and glucocorticoids cooperatively elevate endogenous pEnk gene transcription.

To search for functionally cooperative glucocorticoid and cAMP regulatory elements, rat pEnk genomic clones, isolated by Dr. H. Higuchi, were used to construct plasmids containing the chloramphenicol acetyltransferase (CAT) gene placed under the control of pEnk sequences from bases -2800 to +750 in a promoterless vector. In C6 cells transfected with these constructs, forskolin increased CAT activity 5-17-fold. Unexpectedly, Dex reduced basal and forskolin-stimulated CAT activity by up to 10% and 48%, respectively. These results suggest that the sequences tested contain, in addition to cAMP-regulatory element(s), a negative glucocorticoid regulatory element (GRE), while the expected positive or cooperative GRE may be outside this region.

450.8

EXPRESSION OF NEUROPEPTIDES AND NEUROTROPHIC FACTORS IN THE MAMMALIAN TESTIS. H. Persson, C. Ayer-LeLievre, A. Ericsson, T. Hökfelt, L. Olson, M. Pelto-Huikko, J.F. Rehfeld, M. Ritzen, M. Schalling, O. Söder and M. Villar (SPON: R. Elde) Dept. of Chemistry1, Histology2, Endocrinology3, Karolinska Institute, Stockholm, Sweden. Dept. of Clinical Chemistry, Rigshospitalet, Copenhagen, Denmark.

Expression of the gene encoding the neurotransmitter/neuromodulator cholecystokinin (CCK) is demonstrated in testis of several different species. In situ hybridization revealed CCK mRNA expressing cells in the peripheral parts of the seminiferous tubule. Biochemical identification showed that the majority of preproCCK products in the testis represented proCCK. Immunofluorescence studies revealed CCK-like peptides primarily in spermatocytes/spermatids/spermatozoa of mouse, rat and monkey and immunoelectronmicroscopy on the monkey testis demonstrated CCK-like immunorectivity in the acrosomal granule of spermatids and spermatozoas. Hence, our results suggest that CCK peptides can be released during the acrosome reaction and thus may be of importance in the fertilization process. In addition, the genes encoding the neurotrophic factor β-nerve growth factor and its receptor were both shown to be expressed in the testis. NGF mRNA and protein were found in male germ cells whereas the NGF receptor was expressed in Sertoli cells. Evidence will be presented that expression of NGF and its receptor is controlled by gonadotrophic hormones.

450.9

EXPRESSION PATTERN OF NEUROPEPTIDE GENES IN TRANSGENIC MICE. D.A.Carter*, H.L.Ang* and D.Murphy*. (SPON: P.Wong). Institute of Molecular and Cell Biology, National University of Singapore, Singapore 0511.

Neuropeptide gene regulation has been studied in transgenic mice; bovine transgene expression is detected using species-specific mRNA and peptide probes. A 13.4 kb segment of bovine genomic DNA containing the vasopressin (VP)coding region and 7 kb of upstream sequence was expressed in the hypothalamus (low level) and ovary (high level) of one transgenic line. Observations throughout the estrous cycle indicate regulation of both transgene-derived mRNA, and VP in the ovary. Ovarian expression, which reflects the bovine pattern of endogenous expression, wasalso found in another line containing a chimeric VP/oncogene construct (1.25 kb of VP upstreamregion linked to the SV40 large T coding sequence).In a third line (4.2 kb of bovine genomic DNA containing the oxytocin (OT) coding region and 0.6 kb of upstream sequence) OT expression was detected in the cerebellum, testis and lung. We have demonstrated expression and regulation of bovine neuropeptide transgenes in mice. Additional control sequences may be required to direct a high level of expression to hypothalamic neurons.

450.10

GENOMIC DNA CLONING AND STRUCTURAL ANALYSIS OF THE PORCINE CHOLINE ACETYLTRANSFERASE GENE. C.M.Sampson* and E.E.Baetge. CNS Research, Bristol-Myers Company Wallingford, CT. 06492. Choline acetyltransferase(ChAT), catalyzes the biosynthesis of acetylcholine and is a specific marker for cholinergic neurons in both the peripheral and central nervous system. Recently, the cDNA's for both Drosophila and Porcine ChAT have been cloned.(Itoh.N. et.al. PNAS.,83:4081, 1986; Berrard.S. et.al PNAS.,84:9280, 1987). To begin studies aimed at delineating DNA elements responsible for targeting gene expression to cholinergic neurons we have isolated genomic clones corresponding to the porcine ChAT gene. Overlapping oligos specific to the 5' untranslated region of the ChAT cDNA sequence,(Berrard, S., et al, 1987),were employed as probes to isolate 3 clones from a porcine genomic library. A 2.7 Kb Sst 1 genomic fragment was shown by Southern analysis to hybridize to oligonucleotides specific to the first 50 bp of untranslated sequence as well as the first 50 bp of coding sequence beginning at the initiation codon. Approximately 600 bp of the 2.7 kb Sst I fragment was sequenced revealing the ATG start codon and 20 AA's before diverging at a 5' splice donor sequence. Proceeding 37 bp from the ATG start into the untranslated region, the sequence again diverges at a 3'splice junction. The intron at this junction spans approximately 2,225 bp where it rejoins the next exon at nucleotide 38 in the cDNA. This exon covers a distance of 177 bp where it is interrupted by a 3' splice sequence. Primer extension analysis using an end-labeled oligonucleotide complementary to nucleotides 24-53 in the cDNA, resulted in an extension product of 600 bases in length, indicating that at least 400bp of 5' flanking sequence remain before the end of the gene.

450.11

DEVELOPMENTALLY SPECIFIC ENHANCERS IN THE α AND β SUBUNIT GENES OF THE MOUSE MUSCLE NICOTINIC ACH RECEPTOR. C.A. Prody, V. Shah,* and J.P. Merlie. Dept. of Pharmacology, Washington Univ. Sch. of Med., St. Louis, MO 63110.

The four subunits of the nicotinic acetylcholine receptor are coordinately regulated during skeletal muscle development. To define DNA sequences involved in the transcriptional regulation of the α and β subunit genes, the 5' flanking regions were fused to the chloramphenicol acetyl transferase (CAT) gene and transfected into C2 mouse myogenic cells. CAT activity was obtained in C2 myotubes, but not myoblasts when the -249 to -18 DNA segment relative to the translational start site was used in both orientations in conjunction with the SV40 early promoter. Further deletions resulted in decreased CAT activity, with a 40 bp fragment, 85% identical to the enhancer in the chick α subunit gene, giving 25% of the activity obtained with the long fragment. Relatively low CAT activity was observed in myotubes when β sequences from -890 to -1 relative to the translational start site were used; however, when the -156 to -1 region was fused to CAT, a 5 fold greater CAT activity was seen in myotubes, with low activity in myoblasts. This higher activity was also obtained when a -156 to -70 DNA fragment was used in both orientations with the SV40 promoter. Thus, there appear to be developmentally specific enhancers in both the α and β subunit genes, and a putative negative regulatory element in the β subunit gene.

450.12

STRUCTURAL AND FUNCTIONAL ANALYSIS OF THE CHICK MUSCLE ACETYLCHOLINE RECEPTOR γ-SUBUNIT GENE. H.-T. Jia*#, H.-J. Tsay*#, M. Ballivet*&, and J Schmidt#. #Dept. of Biochemistry, State University of New York at Stony Brook, Stony Brook NY 11794, and &Dept. of Biochemistry, Sciences II, University of Geneva, 1211 Geneva, Switzerland.

The coding regions of the chick muscle acetylcholine receptor δ- and γ-subunit genes are separated by only 743 bp (Nef et al., Proc. Natl. Acad. Sci. U.S.A. 81, 7975, 1984) likely to comprise regulatory elements responsible for the specific expression of the γ-subunit gene. This linker was sequenced, and the single γ-subunit transcription start site localized to position -57 upstream of the translation start site. An inspection of the sequence revealed neither canonical promoter elements nor any obvious similarities to the enhancers present in the α- and δ-subunit genes (Wang et al., Neuron 1, 527, 1988; and unpublished work). We have therefore begun a functional analysis. A 970-bp stretch upstream of the γ-translation start site and including the 12th exon of the δ-subunit gene was fused to the structural gene coding for chloramphenicol acetyltransferase. This construct was expressed at high levels after transfection into C2C12 myotubes, but not in myoblasts. 5' truncation to position -317 upstream of the transcription start site was tolerated; further deletions to -86 led to inactivation. Precise delineation of elements responsible for specific expression is in progress.

450.13

MULTIPLE MRNAS FOR THE TYPE I CORTICOSTEROID RECEPTOR IN RAT HIPPOCAMPUS. P. D. Patel, T. G. Sherman, R. C. Thompson, S. P. Kwak, H. Akil, and S. J. Watson. Mental Health Research Institute, University of Michigan Medical School, Ann Arbor, MI 48109.

We have recently cloned a cDNA for the type I, mineralocorticoid receptor (MR)-like, binding protein from rat hippocampus (Patel et al., *Neurosci. Abst.*, V. 14, 1988). Comparison to human MR cDNA (Arriza et al., *Science*, 237:268, 1987) demonstrates uniformly high homology throughout, except in the 5'-untranslated (5'-UT) region. RNase protection of cRNA with hippocampal mRNA revealed 5'-UT sequence heterogeneity, suggestive of multiple mRNAs. To determine the source of this observation, a genomic fragment spanning the 5'-translated/flanking regions of the rat MR gene was isolated and partially characterized. Sequences were found corresponding to the 5'-UTs for both the rat (β) as well as the human (α) MR cDNAs, and appear to exist as distinct exons separated by approximately 2 kb, situated more than 4 kb upstream of an exon coding for the N-terminal domain. Both the α and β 5'-UT exons display consensus donor sites for proper splicing to produce the observed mRNAs. Taq polymerase chain reaction is being used to determine if the homolog of the human MR 5'-UT is expressed in mature rat MR mRNA.

It it not yet known what role alternate 5'-UT sequences play in expression of the MR. Quantitation of α and β forms does not reveal a clear tissue specific distribution, although there is a tendency for the β form to be expressed at higher levels in CNS tissues. Potential correlations may be found with development or in the stability/translatability of the variant mRNAs.

450.14

IN VITRO TRANSLATION AND MITOCHONDRIAL TARGETING OF HUMAN MONOAMINE OXIDASE (MAO). C. Titlow, J. Hendrick, and X. O. Breakefield. Program of Neuroscience, Department of Neurobiology, Harvard Medical School, Boston, MA 02115

MAO is an enzyme in the metabolic pathway for neurotransmitters such as dopamine, norepinephrine, and serotonin. We have placed a full length 2.0 kb cDNA clone coding for human MAO-A into a Bluescript vector to allow transcription of sense message under control of a T7 promoter. This RNA has been translated in vitro with a rabbit reticulocyte lysate system using ^{35}S-methionine to label the polypeptide. SDS polyacrylamide gel electrophoresis of the translation products revealed a major band with a molecular weight of 66 kD. This band comigrated with authentic human MAO-A (provided by Walt Weyler). In addition, the translation product could be specifically immunoprecipitated with sheep antiserum prepared against MAO-A purified from human placenta (provided by John Pintar). Other experiments demonstrated that ^{35}S-MAO-A could be targeted to rat liver mitochondria. Current studies are underway to determine which regions of the MAO-A molecule are responsible for this targeting. (Supported by NIH grant NS21921)

450.15

PUTATIVE GENOMIC CLONES FOR HUMAN MAO A AND B. R. M. Denney and D. Pizzo*. Dept. of Human Biological Chemistry and Genetics, University of Texas Medical Branch, Galveston, TX 77550.

Human monoamine oxidases (MAO) A and B are homologous, X-linked enzymes which oxidize catecholamine and indoleamine neurotransmitters. We have screened a genomic DNA library (lambda Charon 35 vector; American Type Culture Collection) enriched in X chromosome-specific sequences with either (a) a synthetic oligonucleotide corresponding to a highly-conserved, 59-base pair sequence encoding the FAD-binding site of human MAO A and B (58/59 residues identical; Bach et al., PNAS 85:4934,1988), or (b) segments of cDNA isolated in this laboratory and corresponding ca. to nucleotides 180-1217, and 1218-1750 (MAO A cDNA) and nucleotides 1-249, 250-1291, 1292-2700 (MAO B cDNA). Most genomic clones hybridized strongly to both MAO A and MAO B cDNA fragments. Under the same hybridization conditions, MAO A and MAO B cDNAs hybridized significantly, but weakly, to each other. Therefore, strong hybridization of both MAO A and B cDNAs to individual genomic fragments may indicate that sequences related to MAO A and B are very closely linked (<20 kb apart) in human genomic DNA. [Supported by NS 19543]

450.16

EFFECT OF SECOND MESSENGER ACTIVATION ON EXPRESSION OF TH-CAT CONSTRUCTS INTRODUCED INTO MAMMALIAN CELL LINES. Joanne M. Carroll[1], Linda Hail*[1], Howard M. Goodman*[2], Tong H. Joh[1] [1]Dept. of Neurology and Neuroscience Cornell Univ. Med .College New York, New York 10021 and [2]Dept. of Molecular Biology Mass. Gen. Hospital Boston, MA 02116

Treatment of primary cultures of chromaffin cells with forskolin or TPA results in increased steady state levels of TH mRNA (Evinger et al., 1987). To determine if these agents act at the gene level, a fragment from the 5' end of the rat gene containing bases -151 to +27 was fused to the bacterial CAT reporter and introduced into C6 glioma and Ltk cells for transient assay by the calcium phosphate technique. Expression of the THCAT constructs was readily observed in several nonneuronal cell lines indicating that elements conferring tissue specific expression lie outside this region. The basal levels of expression differed in these two cell lines. While CAT activity measured in the C6 cells was 2 fold over promoterless plasmid controls, levels in L cells were barely above this background. However, when the transfectants were treated with activators of adenylate cyclase (forskolin 10 μM) and protein kinase C (TPA 100nM), the levels under induced conditions were comparable for the two cell lines. In the presence of forskolin, CAT activity in transfected C6 cells was increased by 4-5 fold. TPA increased expression by 1.5-2 fold. These results suggest that the activation of second messenger systems acting through cyclic AMP and protein kinase C leads to induction of TH transcription and that sequences residing within 151 bp of the transcription start site are sufficient to mediate these inductions. (Supported by MH24285-14 and grant from HoeschtAG to MGH.)

450.17

ISOLATION AND CHARACTERIZATION OF THE GENE ENCODING MURINE TRYPTOPHAN HYDROXYLASE. J. Stoll* and D. Goldman. Laboratory on Clinical Studies, National Institute on Alcohol Abuse and Alcoholism, Bethesda, MD 20892.

A full-length tryptophan hydroxylase cDNA was isolated from P815 cells, a mouse mastocytoma cell line. This tryptophan hydroxylase cDNA we isolated from mouse mastocytoma differs from pineal cDNA's previously cloned from rat and rabbit by the insertion/duplication of three amino acids near the N-terminus of the predicted amino acid sequence. Bacterial cells containing this cDNA fused to the gene encoding S. aureus protein A exhibit tryptophan hydroxylase activity. Different portions of the cDNA were fused to protein A and the tryptophan hydroxylase activity measured to delimit the portions of the gene which encode catalytic activity. An internal region encompassing approximately two-thirds of the gene is required. This region is highly similar in sequence to the other aromatic amino acid hydroxylases. The cDNA recognizes mRNA's from pineal, duodenum, and brainstem when used to probe Northern blots. The cDNA was used as a hybridization probe to isolate genomic clones from a library. These clones are used to reveal the structure of the gene. Characterization of the gene will allow the determination of elements important in the regulation of tryptophan hydroxylase.

450.18

CLONING AND CHARACTERIZATION OF THE RAT GLUTAMINE SYNTHETASE GENE, AND FULL LENGTH CDNA. J.F. Mill*, K.M. Mearow, and E. Freese. Lab. Molec. Biol., NINDS, NIH, Bethesda, MD 20851.

Glutamine synthetase (GS) catalyzes the synthesis of glutamine from glutamate and ammonia. GS plays a central role in nitrogen metabolism and ammonia detoxification in the central nervous system. Our interest in GS expression is due to its complex regulation by glucocorticoids, cAMP, insulin, and thyroid hormone, and its differential expression by astrocytes in the CNS.

We screened a rat cDNA library which was enriched in full length clones. A clone of 2.6 Kb in length was selected through complimentarity to a previously cloned GS cDNA which was not full length (Mearow et al., 1989). Sequence analysis of this clone shows it to be GS and to contain the 5' end of the message.

This cDNA clone was used to isolate the GS gene from a rat genomic library constructed in Charon 4a. The 16 Kb insert was subcloned as 4 Eco RI fragments. Sequence analysis has determined the first two exons and the 5' flanking region. The first exon extends 122 bp, from the transcriptional start site to upstream of translational start site. The first intron is approximately 2 Kb in length and is followed by the second exon which continues for 176 bases.

Sequences with homology to the glucocorticoid and cAMP response elements, and the AP-2 transcriptional activator have been found upstream from the start site for transcription. These regions may act as binding sites for the trans-acting factors responsible for transcriptional regulation of GS.

450.19

GLUTAMATE RECEPTORS MEDIATE IMMEDIATE-EARLY GENE EXPRESSION *IN VIVO*. R.A.Molinar-Rode, T.Curran* and J.I.Morgan*. Depts. of Neurosciences and Molecular Oncology, Roche Institute of Molecular Biology, Roche Research Center,Nutley, NJ 07110.

Cellular immediate-early genes are defined by their similarities with regulatory genes of eukaryotic viruses, notably their rapid induction by extracellular stimuli, even in the absence of protein synthesis. The products of three such genes, c-*fos*, c-*jun* and *jun*-B, are components of transcription factor AP-1, and may act as nuclear third messenger molecules, coupling stimulation to long-term transcriptional responses. Here we describe the temporal pattern of expression of a number of cellular immediate-early genes in mice (using Northern blot analyses). after activation of various classes ofexcitatory amino acid (EAA) receptors. BRAIN: Pentylentetrazole (PTZ), which activates the NMDA-type of EAA receptor, causes a rapid and transient increase in c-*fos* and *jun*-B mRNA levels in brain. In contrast, c-*jun* rises and remains elevated for at least 6 h after seizure. Kainate (KAI) stimulation results in a rapid, but much protracted, induction of c-*fos* and *jun*-B. However, c-*jun* is not strongly induced by this agent. LUNG: PTZ causes a minor, transient increase of c-*fos* and *jun*-B mRNA in lung. However, c-*jun* is constitutively high, both before and following treatment. KAI, in contrast, produces a large increase in c-*fos*, which remains elevated for at least 2 h. c-*jun* transcripts are also rapidly induced by KAI and peak 45 min after administration. decreasing gradually thereafter. The response of *jun*-B is similar to c-*fos*. GUT: PTZ and KAI caused small increases in c-fos, c-jun and *jun*-B expression. However, basal mRNA levels of all three are higher in gut than either lung or brain. We establish here that activation of EAA receptors *in vivo* results in a tissue and ligand specific induction of three of the immediate-early genes.

450.20

DIFFERENTIAL EXPRESSION OF ARPP-16 AND ARPP-19, TWO CLOSELY RELATED cAMP-REGULATED PHOSPHOPROTEINS. Atsuko Horiuchi*, Jean-Antoine Girault, Angus C. Nairn and Paul Greengard. Laboratory of Molecular and Cellular Neuroscience, The Rockefeller University, 1230 York Avenue, New York, NY 10021

ARPP-16 and ARPP-19 are two cAMP-regulated phosphoproteins (Mr=16,000 and Mr=19,000, respectively). Sequence analysis of the cloned cDNAs and the purified proteins indicate that the two proteins differ from each other by 16 additional amino acid residues on the NH_2-terminus, which are present only in ARPP-19. Comparison of the base sequence of ARPP-16 and ARPP-19 cDNAs reveals extensive segments of total identity in the translated and untranslated regions. The structure of the genes encoding for these two proteins has been analyzed by Southern hybridization of genomic DNA, using a variety of probes corresponding to the regions common to the two cDNAs and the regions specific to each of them. The regional, ontogenetic and phylogenetic distribution of ARPP-16 and ARPP-19 has been studied by Western blotting. ARPP-19 was found in the brains of all the vertebrate species studied and, at various levels, in all the tissues of adult rat. ARPP-19 was also present at high levels in several malignant cell lines and in normal embryonic tissues where it decreased during development. In contrast ARPP-16 was detected only in some specific neurons of the dopamine-innervated regions of the cerebral cortex and the basal ganglia, which are known to possess the D1 dopamine receptor. ARPP-16 was phylogenetically recent, being found only in birds and mammals and appeared late during ontogenesis, during the post-natal period. These observations suggest that the regulation of the expression of ARPP-16 and ARPP-19 is very different. ARPP-19 is expressed in all cell types especially when they are not fully differentiated, while ARPP-16 appears to be expressed only in neurons which possess the dopamine D1 receptor.

450.21

REGULATORY ELEMENTS IN THE 5' FLANKING REGION OF THE TYPE II SODIUM CHANNEL GENE CONFER CELL SPECIFIC EXPRESSION *IN VITRO AND IN VIVO* R. A. Maue, K. Ebert*, M. Low*, and G. Mandel*, Division of Molecular Medicine, New England Medical Center, Boston, MA 02111, and Department of Anatomy and Cellular Biology, Tufts University School of Veterinary Medicine, N. Grafton, MA 01536

The mammalian sodium channel genes exhibit tissue specific differences in their expression. To identify sequences in the brain type II gene required for its tissue specific pattern of expression, we have isolated a rat genomic DNA fragment containing approximately 1.1 kb of the 5' flanking region of this gene and have constructed a fusion gene between this fragment and the prokaryotic reporter gene chloramphenicol acetyltransferase (CAT). The expression of this fusion gene in transiently and stably transfected cells was significantly higher in neuronal cell types, such as rat pheochromocytoma (PC12) and human neuroblastoma (NB-5) cells, than in a variety of other cell types, including rat glioma (C6), rat myoblasts and myotubes (L6), mouse fibroblasts (3T3), and human hepatoma cells (HepG2). We subsequently introduced the same fusion gene into transgenic mice and found that the 5' flanking region is sufficient to direct tissue specific expression *in vivo*. When assayed in adult mice, high levels of CAT activity were detected in the brain, with no detectable expression in the liver, kidney, heart, skeletal muscle, or eye. Deletional studies in transiently and stably transfected cells indicate that the regulation of the type II gene depends on both positively- and negatively-acting elements in the 5' flanking region. Further localization and characterization of these elements will provide a foundation for elucidating the molecular mechanisms regulating the sodium channel gene family.

mRNA REGULATION V

451.1

THE FAMILY OF HEAT SHOCK PROTEIN 70 mRNAs ARE DIFFERENTIALLY INDUCED IN RAT CEREBELLUM, CORTEX, AND NON-NEURONAL TISSUES. E.K. Miller*, J.D. Raese and M.R. Morrison-Bogorad, Depts of Neurology, Biochemistry and Psychiatry, U.T.-Southwestern, Dallas, Texas, 75235 and the Schizophrenia Res. Ctr., V.A. Medical Ctr., Dallas, Texas, 75216.

Oligonucleotides were synthesized to hybridize to either the inducible HSP70 mRNA, the constitutively expressed cognate HSC70 mRNA, or the glucose-regulated protein, GRP78 mRNA. mRNAs encoding GRP78 (3.05 kb), and HSC70 (2.55 kb) were detected in all tissues. No HSP70 mRNA was detected in control tissues. After heat shock or amphetamine-induced hyperthermia, two HSP70 mRNAs (3.53 and 3.05 kb) were induced. These mRNAs were quantitated by slot blot analysis relative to the levels of 18S RNA. There were dramatic tissue-specific differences. The induction of HSP70 mRNAs was always several fold greater in non-neuronal tissues than in brain. In cerebellum, HSP70 mRNA induction was always several fold higher than in cortex. Both treatments also increased HSC70 mRNA levels, again more in cerebellum than in cortex. In amphetamine-treated animals, temperatures above 40°C were required for HSP70 mRNA induction but not for induction of the other mRNAs. This data suggests that neuronal tissue may be more susceptible to cellular stress than non-neuronal tissue. Furthermore, cortex may be more vulnerable than cerebellum. Supported by NIH AG08013 (MRM-B and JDR), NIH HD14886 and the Leland Fikes Foundation.

451.2

IN SITU QUANTITATION OF HEAT SHOCK 70 mRNAs IN RAT CEREBELLUM AFTER AMPHETAMINE-INDUCED HYPERTHERMIA. M.R. Morrison-Bogorad, K. Groshan*, E.K. Miller*, and J.D. Raese, Depts of Neurology, Biochemistry and Psychiatry, U.T.-Southwestern Medical Center, Dallas, Texas, 75235, and the Schizophrenia Res. Ctr., V.A. Med Ctr, Dallas, Texas, 75216.

We have synthesized oligonucleotide probes that specifically recognize the stress-induced (HSP70) or the constitutively expressed (HSC70) heat shock mRNAs. The two mRNAs were quantitated by in situ hybridization in different cell types of rat cerebellum relative to total poly(A) and 18S RNA. No HSP70 mRNAs were found in control rat cerebellum. HSC70 mRNAs were present at similar relative levels in Purkinje and granule cells, but the mRNA was sparse in all non-neuronal cells. After amphetamine-induced hyperthermia, HSP70 mRNA was dramatically induced in non-neuronal cells (pial cells, oligodendrocytes and, possibly, Golgi epithelial cells). Induction of the HSP70 mRNA was much less pronounced in neurons. Granule cells had the highest levels, and Purkinje cells were least responsive. These results show that the magnitude of the HSP70 response to amphetamine-induced hyperthermia differs in different brain cells and different neuronal classes. The extent of induction of this, or other, stress-related proteins may be important in determining the vulnerability of different brain cell populations to environmental or physiological stress. Supported by NIH HD P50-AG08013 (MRM-B and JDR), NIH 14886 and Leland Fikes Foundation (MRM-B).

451.3

REGULATION OF TRANSLATION BY STEROIDS: MYELIN PROTEIN mRNAs. J. M. Verdi*, S. W. Grant*, AND A. T. Campagnoni. (SPON: C. Clemente) U.C.L.A. Medical School, 760 Westwood Plaza, Los Angeles, CA 90024.

The translation of several myelin protein synthetic mRNAs were examined in reticulocyte lysates and myelin basic protein (MBP) mRNAs were found to translate less efficiently than other mRNAs tested. Among the transcripts encoding four different isoforms of MBP the 18.5kDa MBP mRNA translated more efficiently than the others. This indicates that coding regions of the mRNA, in addition to the untranslated regions, can influence the efficiency with which mRNAs are translated. The role of the 7me-guanosine cap in the translation of myelin protein mRNAs was also examined. Like most mRNAs, the CNPase message was translated more efficiently when it was capped. In contrast, MBP mRNAs translated 1.6x better when they were uncapped. Since the MBP mRNAs are capped in vivo, this places them at a further translational disadvantage compared to other myelin protein messages. We also observed that steroids could modulate the efficiency with which myelin protein mRNAs were translated. When MBP and PLP mRNAs were translated in the presence of 10^{-8} M hydrocortisone, synthesis of these proteins was stimulated two fold; but the translation of CNPase was inhibited 60%. Steroids had no effect on the translation of several other mRNAs. Thus, steroids may alter the translational efficiency of myelin specific mRNAs by increasing the efficiency of poorly translating messages such as MBP, and inhibiting the translation of favored mRNAs like CNPase, thereby altering the levels of polypeptides that are synthesized. Such findings may provide a molecular explanation for the changes in the proportions of these proteins known to occur with development. (Supported by NIH grants NS23022 and NS23322).

451.4

HEAT SHOCK RESPONSE IN GERBIL BRAIN AFTER ISCHEMIA -- IN SITU HYBRIDIZATION ANALYSIS. T. S. Nowak, Jr. Lab. of Neuropathology and Neuroanatomical Sciences, NINDS, NIH, Bethesda, MD 20892.

The distribution and induction time course of RNA encoding the 70 kDa stress/heat shock protein (hsp70) was determined in postischemic gerbil brain by in situ hybridization with a ^{35}S-labeled oligonucleotide selective for inducible species of the hsp70 family, and compared with that of immunoreactive hsp70 detected with a monoclonal antibody having similar selectivity. In control animals hsp70 hybridization and immunoreactivity were detected only in ependymal cells. Following 5 min bilateral carotid artery occlusion hsp70 RNA was evident in dentate granule cells within 3 h, with prominent induction in all hippocampal pyramidal cell fields by 6 h recirculation. Within 24 h the signal was greatly reduced in those neuron populations which eventually showed hsp70 immunoreactivity, while hsp70 RNA sequences remained elevated through at least 48 h in CA1 neurons which fail to express the protein. No hsp70 hybridization remained in the CA1 region following the loss of these neurons by 4 d. CA1 neurons vulnerable to ischemic injury thus demonstrate a robust and even prolonged heat shock response at the transcriptional level under conditions in which profound deficits in translational activity may preclude accumulation of immunoreactive protein. In CA3 neurons there was a notable lag between the disappearance of hybridizable RNA at 24 h and the detection of peak immunoreactivity at 48 h, raising the alternative possibility that hsp70 synthesized in CA1 neurons may be similarly inaccessible to the antibody during a period of active stress.

451.5

CONSTITUTIVE HEAT-SHOCK-70 mRNA IN BRAIN IS PRIMARILY NEURONAL AND IS INCREASED BY ESTROGEN IN HYPOTHALAMUS. C.V. Mobbs, K.L. O'Malley[1], A.H. Lauber, and D.W. Pfaff (SPON: Z. Wenzel). Rockefeller Univ., New York, NY, 10021, and [1]Washington Univ., St. Louis, MO 63110.

We previously showed that constitutive heat-shock-70 (hsp70) mRNA is increased by estrogen in uterine secretory cells. In this study we examined if estrogen would increase hsp70 mRNA in the arcuate and ventromedial hypothalamic nuclei (VMN), two areas containing estrogen receptors. Ovariectomized female Sprague-Dawley rats were given implants containing estradiol, or sham implants, and sacrificed 6 hours later. The brains were processed for in situ hybridization, using a 3H-labelled cDNA probe (pRC62) complementary to constitutive hsp70 mRNA. Grains were localized over neurons, with few grains over glia or ependyma. The results, representing 50 cells of each brain region and 50 glial cells from VMN from each animal, are shown below (grains/cell, mean ± SEM).

	VMN	Arcuate	Hippocampus	Glia
No E2	6.9 ± 0.3	4.5 ± 0.5	5.0 ± 0.6	1.0 ± 0.3
6h E2	16.8 ± 0.8	10.2 ± 0.4	6.6 ± 0.5	1.4 ± 0.3

These results indicate that constitutive hsp70 mRNA is found mainly in neurons and is increased by estrogen in hypothalamic nuclei which contain estrogen receptors, but not in hippocampus, which contains few estrogen receptors.

451.6

LOCATION OF THE PROMOTER REGION FOR THE LAMININ B2 GENE IN RAT C-6 GLIOMA CELLS. H. HALEEM-SMITH*, J.R. WUJEK, E. FREESE, P. BURBELO*, AND Y. YAMADA* (SPON: K. BICK). Lab. of Molec. Biol., NINDS, NIH and Lab. of Dev. Biol. and Anomalies, NIDR, NIH., Bethesda, MD 20892.

Laminin, a protein of the extracellular matrix with strong neurite outgrowth promoting activity, is transiently expressed during development of the rodent central nervous system (Letourneau et al., Dev. Biol., 1988, 125: 135). Astrocytes, which stimulate neurite outgrowth *in vitro*, have recently been shown to synthesize and secrete only the B2 chain of laminin (Wujek et al., Neurosci. Abstr. , 1988, 14: 1326). We have investigated the molecular mechanisms that regulate expression of the laminin B2 chain in astrocytes. We made constructs containing the promoter region (-830 to +106) of the B2 chain gene fused to a structural part of the chloramphenicol acetyltransferase gene (Ogawa et al., 1988, J. Biol. Chem. 263: 8384) and examined the promoter activity by transfecting them into rat C-6 glioma cells and F9 teratocarcinoma cells (differentiated F9 cells synthesize abundant quantities of laminin). This first construct exhibited significant activity in both cell lines. Further deletions of the promoter (down to nucleotide -94) were constructed and transfected into F9 and C-6 cells. In both cell lines, all deletion constructs exhibited significant activity. These results indicate that a sequence, located between nucleotides -94 and +106 of the laminin B2 chain gene, is needed for expression in both the rat C-6 glioma and mouse F9 teratocarcinoma cells.

451.7

ALTERNATIVE SPLICING AND POLYADENYLATION OF HUMAN SYNEXIN mRNA. K. Magendzo*, H. B. Pollard and A. L. Burns* (Spon: A. Basile). Lab. of Cel. Biol. and Gen.,NIDDK, NIH Bethesda MD 20892.

Synexin is a calcium dependent membrane binding protein that aggregates adrenal chromaffin granules and acts as a voltage-dependent calcium channel in artificial bilayers. The sequence of human synexin cDNA clones showed that synexin belongs to a family of endonexin II-like proteins, all of which bind to membranes in the presence of calcium (Burns, A.L. et. al., Proc. Natl. Acad. Sci.USA(1989) 86: in press). Northern blot analysis of human liver and adrenal medulla mRNA revealed the existence of two distinct synexin bands of 1.95 Kb and 2.4 Kb. The possibility of alternative splicing was examined by isolating and sequencing several human synexin cDNA clones. Two of the clones analysed contained a longer 3' untranslated region (336 bp) generated by the selection of an alternative polyadenylation site. We have also isolated a cDNA clone containing a presumed exon (66 bp) located at the 5' end region. The deduced aminoacid sequence of this exon introduces 3 acidic aminoacids to an otherwise highly hydrophobic, unique N-terminal domain. The prevelance of these transcripts is being analysed by screening specific regions of human synexin mRNA using the polymerase chain reaction technique and detecting the amplified products with oligonucleotide probes. The functional role of these putative alternative splicing is being investigated.

451.8

CNS-SPECIFIC PROBES FROM SUBTRACTIVE cDNA LIBRARIES. J.R. Hofstetter*, J.J. Wei*, B. Ghetti, J.I. Nurnberger, Jr., and M.E. Hodes* (SPON: B. Azzarelli). Departments of Medical Genetics, Pathology, Psychiatry and Institute for Psychiatric Research, Indiana Univ. Med. Sch., Indianapolis, IN 46223.

To identify morphologically important transcripts specific to CNS neurons, we constructed mouse subtractive cDNA libraries (cerebellum minus frontal cortex and cerebellum minus frontal cortex and liver). We identified several recombinant clones that were apparently brain-specific. We amplified one cloned insert, designated P8, from purified lambda phage DNA using the polymerase chain reaction, cloned it directly into M-13, and sequenced it by the Sanger method. The gene fragment corresponds to a 6.7-kilobase mRNA from mouse cerebellum and cortex. The sequence of the probe does not have homology with any sequences in the Gene Bank data base and contains a potential coding region for 60 residues of a protein.

Based on the sequence, we obtained two synthetic single-stranded probes, thirty nucleotides long. These are being used for in situ transcription and hybridization on mouse brain slices to determine the distribution of P8 in the CNS. We intend to find the extent of cross-species expression, chromosomal origin, and developmental onset of expression in the mouse of this new probe.

Supported by USPHS grant RO1-NS14426.

451.9

WITHDRAWN

451.10

DIFFERENTIAL PROMOTER UTILIZATION GENERATES A NOVEL FAMILY OF RAT NEURONAL PROTEINS P.E. Danielson, S.Forss-Petter, E.Battenberg, R.J. Milner, F.E.Bloom and J.G.Sutcliffe. Research Institute of Scripps Clinic, La Jolla, CA 92037

Four forms of 1B426B mRNAs are generated from 2 genes by alternate transcriptional initiation and subsequent 5' end splicing. The mRNAs are most abundant in cortex and hippocampus, but are present at lower levels in other brain regions. Some forms are found in pituitary>adrenals> spleen, thymus and ovaries, but none is found in any of several other tissues. In situ hybridization shows enrichment in dentate granule cells and polymorphic neurons, and pyramidal neurons of the hippocampus (CA4>CA3>CA1) and neocortical layers 3,5 and 6. 1B426B mRNAs encode 4 distinct apparently secretory and/or membrane proteins of 125, 153, 457 and 485 amino acids that differ from each other only at N and/or C termini. Antisera to synthetic peptide fragments of the common region detect four proteins in brain extracts whose mobilities shift after endoglycosidase treatment, indicating that these are glycoproteins. Form-specific subprobes suggest developmentally regulated cell-specific expression of members of this mRNA family.

451.11

CHARACTERIZATION OF MRNA IN PREPARATIONS OF RAT CORTEX SYNAPTOSOMES. I. J. Weiler, G.S. Withers, C. S. Wallace and W. T. Greenough. Beckman Inst., Depts. Psych. & Cell & Struct. Biol., & Neur. & Beh. Biol. Prog., Univ. IL, Champaign, IL 61820.

Cortical synaptosomes from young (P 18-21) and old rats were prepared in buffers containing cycloheximide and RNase inhibitors (heparin, vanadyl complexes). After samples were taken for EM examination, synaptosome suspensions were lysed and polysomes were collected by ultracentrifugation through 1 M sucrose. RNA was isolated with guanidine isothiocyanate, followed by two precipitations out of guanidine-HCL. Total RNA was electrophoresed in 1% agarose gels containing 2M formaldehyde, and transferred to Gene screen. Radiolabelled oligo-dT probes have demonstrated the presence of polyadenylated mRNA. We are currently engaged in probing further samples for the presence of specific messages, among which are actin, GAP43, MAP2 (microtubule-associated protein), and synthetic oligonucleotides complementary to the sequences of N-CAM and calcium-calmodulin-dependent protein kinase II. Supported by MH35321.

451.12

CORTICOSTERONE REGULATED GENES IN THE HIPPOCAMPUS: REGULATION BY THE TYPE I OR TYPE II GLUCOCORTICOID RECEPTOR. J.N. MASTERS, N.R. NICHOLS, H.H. OSTERBURG AND C.E. FINCH. Andrus Gerontology Center, USC, Los Angeles, CA 90089-0191

We have used corticosterone (CORT) regulated cDNA clones isolated from a rat hippocampal library to analyze steroid and stress-responsiveness in individual rats. The clones include glial fibrillary acidic protein (GFAP), glycerol phosphate dehydrogenase (GPDH) and CR16 which codes for an unidentified gene product expressed in hippocampal neurons; GFAP RNA abundance decreases with CORT while both GPDH and CR16 increase with CORT. The steady state levels of total RNA for the three clones was measured by RNase protection or RNA blots quantified by videodensitometry. In adrenalectomized (ADX) rats, all clones exhibit maximal responses to RU28362 consistent with being regulated by the type II receptor. As the type II glucocorticoid receptor is thought to mediate stress responses, we analyzed the effects of stress on the RNA abundance of these clones in individual rats. The animal groups were 3d ADX, 7d ADX, intact (INT, sacrificed in the AM) and INT + Stress (2 hr stress). GPDH RNA was increased 4-fold in INT rats by vibratory stress. There was no significant difference between INT and ADX groups. In contrast, CR16 and GFAP showed near maximal responses in INT versus ADX rats and no further increase to stress. These data are consistent with GPDH being regulated solely by the type II glucocorticoid receptor as a stress response. CR16 and GFAP may also be regulated by the type I receptor since low AM CORT significantly alters their expression. We plan to confirm the type I component by adding low levels of CORT with Alzet osmotic pumps as well as analyze the expression of these genes during the circadian rhythm. Supported by ONR grant N00014-85-K-0070; NRN is a Brookdale National Fellow.

451.13

ANALYSIS OF mRNAS SHOWING REGION-SPECIFIC DISTRIBUTION IN NONHUMAN PRIMATE CORTEX. K.Chandrasekaran*, M.F. Matocha, S.P.Wise and S.I. Rapoport. Lab. of Neurosci., NIA, Bethesda, MD 20892 and Lab. of Neurophysiology, NIMH, PO Box 289, Poolesville, MD 20837.

It has been proposed (Rapoport, S.I. Rev Neurol, Paris 144:79, 1988) that Alzheimer's disease is a 'phylogenetic' disease afflicting the recently elaborated associative neocortices and their connections to older brain regions. To examine this hypothesis further, we have begun to identify mRNAs that show specific expression in associative neocortical regions of the Rhesus monkey. cDNA libraries were generated in λgt10 using mRNAs prepared from frontal pole and from visual cortex brain areas. Initially 10,000 plaques in the frontal pole cDNA library were screened by subtractive and differential hybridization. 31 cDNA clones were identified that hybridized specifically with the total cDNA probe prepared from the frontal pole but not with the probe from the visual cortex. A secondary screening confirmed that several of these clones retained a differential hybridization pattern. The results on the identification and characterization of these clones will be presented.

451.14

DIFFERENTIAL RECOMBINANT cDNA CLONES ISOLATED FROM A SUBTRACTED LIBRARY CONSTRUCTED FROM A QUIESCENT NEURONAL CELL LINE. P. Crisanti*, L. Bidou* and B. Pessac. Eq. de Neurobiologie Cellulaire et Moleculaire, CNRS LP 3101, 67 rue M. Günsbourg, 94205 IVRY S/SEINE (France).

In order to investigate if the negative control of cell proliferation can be correlated with the expression of specific genes, we have constructed a subtracted cDNA library from a quail neuroretina cell clone immortalized with the RVS mutant ts-NY-68 (a sister clone is described in Nature (302) 616-618). This clone is thermosensitive for transformation and multiplication; indeed, cells multiply at 36°C while they are quiescent 48 hours after a shift up to 41.5°C. At this time point, renewal of fetal calf serum containing culture medium does not induce DNA synthesis. However, cells in S phase appear 6 hours after a shift-down to 36°C. The cDNA library was constructed from cells that had become quiescent 48 hours after a shift up. To enrich the cDNA library in transcripts which might be expressed at a low level, single strand cDNA synthesized from poly(A)$^+$ mRNA of cells cultured at 41°C were subtracted by liquid hybridization with an excess of poly(A)$^+$mRNA of cells cultured at 36°C. Thus, a 13-fold enriched cDNA library containing 8000 clones was obtained. This library was differentially screened with "41°" and "36°" cDNA probes both subtracted with poly(A)$^+$mRNA of cells cultured at 36°C. About twenty clones hybridized to poly(A)$^+$mRNA of quiescent cells cultured at 41°C and not to poly(A)$^+$mRNA of cells cultured at 36°C.

We are currently investigating the expression of the mRNAs corresponding to the cDNA clones in various quiescent nervous and non-nervous quail tissues. In parallel, partial sequences of the cDNAs is being studied.

451.15

A NOVEL IN VITRO cDNA AMPLIFICATION METHOD: LINEAR AMPLIFICATION OF RAT CEREBELLAR cDNA. R.N. Van Gelder*, M. von Zastrow*, W.C. Dement, J.D. Barchas, J.H. Eberwine* (SPON: S. Leff). Basic Sleep Research Laboratory and Nancy Pritzker Laboratory of Behavioral Neurochemistry, Stanford U. School of Medicine, Stanford, CA 94305.

In order to isolate and characterize brain nucleus- and cell-specific messages from very small amounts of neural tissue, we have developed a novel in vitro cDNA amplification method. cDNA is prepared from total RNA using a synthetic primer which includes the T7 bacteriophage RNA polymerase promoter site. Addition of T7 RNA polymerase generates anti-sense, amplified RNA (aRNA). We have achieved up to 80-fold molar amplification of rat cerebellar RNA using this technique. The aRNA matches the parent cDNA exactly in size distribution. The amplified material is of high complexity as demonstrated by R_0t analysis and binding of aRNA to Northern and Southern blots. We are able to detect specific, low abundance messages in the amplified material. We anticipate this technique will be of great utility in cloning and characterizing messages of interest from limited amounts of very heterogeneous neuronal tissue.

INGESTIVE BEHAVIORS V

452.1

SOYBEAN TRYPSIN-INHIBITOR DECREASES INGESTION OF MILK IN NEONATAL RATS. A. Weller, G.P. Smith and J. Gibbs. Bourne Lab., New York Hospital-Cornell Medical Center, White Plains, NY 10605.

Trypsin-inhibitor (TI) is a potent releaser of endogenous CCK in adult rats (Liddle et al., Gastroenterol. 87:524, 1984). Assuming a similar effect in the young rat, TI was used to test the hypothesis that endogenous CCK would reduce the intake of milk diet by neonatal rats just as exogenous CCK inhibits intake (Robinson et al., Am. J. Physiol., 255:R14, 1988). Nondeprived rat pups, 10-12 days old, received an intragastric infusion of TI (100 mg/1 ml saline) or isotonic saline. 15 min later they were placed on a towel soaked with commercially available 'Half and Half'. TI significantly reduced the percent body weight gain in two separate replications: means= 1.28% vs 0.69% and 1.29% vs 0.034% for the saline-TI comparisons in the two replications ($p<.05$; N=8 rats per infusion group in each replication). These results suggest, but do not prove, that TI decreased intake by releasing CCK from the small intestine.

452.2

PSYCHOPHARMACOLOGY OF CONSUMMATORY RESPONSE EXTINCTION. C. F. Flaherty, K. Hrabinski*, J. Reese*, and J. Alvarez*. Psychology Dept., Rutgers University, New Brunswick, NJ 08903.

Anxiolytics may or may not retard extinction of consummatory behavior (empty tube licking) following access to water (e.g. Miczek & Lau, Psychopharm., 42:263269, 1975; Soubrie, et al., Psychopharm., 59:95-100, 1978). Drug effects on empty-tube licking following training with a 32% sucrose solution are reported here. Rats kept at 82% of their free-feeding weight were allowed daily (10 days) five minute access periods to 32% sucrose. A twenty-minute extinction session (empty tube) was administered on the eleventh day. All drugs were administered acutely in extinction.

Chlordiazepoxide (8 mg/kg) and sodium amobarbital (17.5 but not 20 mg/kg) enhanced initial resistance to extinction. Buspirone (0.5 and 2.0 mg/kg) had little effect, but tended to facilitate extinction. Cyproheptadine (3 and 6 mg/kg) had no effect on extinction. These results tend to parallel the effects of the same drugs on negative contrast, where rats are shifted from 32% to 4% sucrose. However, in the contrast procedure, CDP is maximally effective late rather than early in the 'post-shift' period, and cyproheptadine had potent anti-contrast effects. Ongoing experiments involve the comparative effects of amphetamine, imipramine, methysergide, ritanserin, and ethanol.

452.3

EFFECTS OF CORTICOSTERONE IN DRINKING WATER ON NATURAL FEEDING PATTERNS. M.Yamamoto*, D.Tempel and S.F.Leibowitz (Spon:G.Brennan). Rockefeller University, N.Y.,N.Y. 10021

Corticosterone(CORT) is known to be involved in natural feeding processes. Adrenalectomy(ADX) results in a suppression specifically of carbohydrate(C) intake, at the onset of the nocturnal cycle when ingestion of this macronutrient is normally high. C ingestion is restored by peripheral injection or central implants of CORT. In the present study, we provided ADX animals with free access to a CORT-supplemented saline solution(200ug/ml), or vehicle (0.9% saline), 17 or 3 hrs prior to dark onset, and recorded macronutrient intake during the 1st hr of the dark cycle. Results indicate that animals consumed similar amounts of the CORT and vehicle solution. CORT presentation, 17 or as little as 3 hrs before dark onset, resulted in a significant increase in 1-hr C intake(+2.7 Kcal, p 0.05) over vehicle baseline, with no change in fat or protein intake, and a tendency toward an increase in total food intake. In addition, a strong positive correlation(r=0.73, p 0.05) was observed between CORT intake(mls) and 1-hr C intake(Kcal) at dark onset. No similar relation was observed with vehicle consumption. These effects of CORT were observed after the ingestion of as little as 5-6 mls(approximately 1 mg CORT) during the 3-hr pretreatment period, and support a possible role for the glucocorticoid in natural feeding processes.

452.4

RACLOPRIDE DECREASES INTAKE OF SUCROSE IN RATS AS EARLY AS POSTNATAL DAY 14. G.P. Smith, C. Gayle*, J. Gibbs, R.D. Shindledecker* and S.H. Ackerman. E.W. Bourne Lab, NY Hosp-Cornell Med. Ctr., White Plains, NY 10605.

Raclopride, a D-2 dopaminergic antagonist, decreases the sham feeding of sucrose in adult rats (ID_{50}=150 ug-kg^{-1},ip, Schneider et al, 1988). To determine the ontogeny of this effect, pups were removed from the litter on postnatal days 7,14 and 21. Some pups were equipped with an anterior, sublingual catheter (Hall, 1979). Four h later, the intake of 10% sucrose was measured in one of two 20-min tests in a warm, humid environment: (1) continuous, sublingual, intraoral infusion or (2) independent ingestion in which a pup ingested 10% sucrose from tissues in the bottom of a test beaker. A dose of raclopride or saline alone was injected ip 15 min prior to an intake test. Each pup was tested only once. Raclopride was significantly more potent for decreasing intake in the independent ingestion test than in the intraoral infusion test after day 7 (Table).

POTENCY OF RACLOPRIDE (ID_{50}, ug-kg^{-1}, ip)

DAY	INTRAORAL CATHETER	INDEPENDENT INGESTION
7	>3333	>3333
14	1714 - 3429	109
21	>1333	83

Although the reason(s) for the differential potency in the 2 tests is not clear, the results suggest that D-2 receptor activity is necessary for the positive reinforcing effect of sucrose on independent eating as early as postnatal day 14.

452.5

CENTRALITY OF HISTAMINE AND CRF IN PEM-INDUCED SUPPRESSION OF FOOD INTAKE. J.D.Dunn, S.Dodds,* M.Weber,* D.Hampton* and L.P.Mercer.* Depts. of Anat. and Biochem., Sch. Med., Oral Roberts Univ., Tulsa, Ok 74171.

We have recently put forth the suggestion that histaminergic stimulation of corticotropin releasing factor (CRF) is central to the suppression of food intake (FI) observed in protein energy malnutrition (PEM). Brain histamine (HA) as well as histidine (HI) levels are increased during PEM, and PEM-induced suppression of FI is partially ameliorated by administration of HI decarboxylase.

To further pursue our hypothesis, we evaluated the effect of PEM on several parameters of pituitary-adrenal function. Additionally we assessed the effect of electrolytically ablating the paraventricular nucleus of the hypothalamus (PVN) on FI of rats fed a low protein diet (8% casein). In all studies adult male rats were assigned to treatment groups and studies were conducted according to a randomized block design. Control diets contained 25% casein. Body weight and FI were recorded daily and blood samples for Cpd B (RIA) were collected via rapid decapitation.

Both AM and PM non-stress plasma corticosterone (Cpd B) levels of rats fed a PEM diet were higher (P<0.05) than those of rats fed a control diet. Concomitant with decreased FI and elevated Cpd B levels, PEM rats showed a 75% increase in hypothalamic HA concentrations (RIA) compared to rats fed a control diet. Significantly, PVN ablation abolished the PEM-induced suppression of FI observed in controls.

Collectively these data, taken together with our previous studies, offer considerable support for our hypothesis that histaminergic stimulation of CRF is involved in PEM-induced suppression of FI.

452.6

FETAL DIETHYLSTILBESTROL (DES) EXPOSURE: A POSSIBLE RISK FACTOR IN THE DEVELOPMENT OF ANOREXIA NERVOSA. C.R.Gustavson,J.C.Gustavson*,K.L.Noller*,L.J.Melton,III*, P.C.O'Brien*,A.J.Pumariega*. Psych. Ethology Lab., Univ. of Texas Med. Br. Sch. of Med., Gal., TX 77550, & DESAD Project, Mayo Foundation, Rochester, MN 55905.

From data collected using rats as a model, & conditioned taste aversions as a measure of malaise, we proposed a neuroethological model of anorexia nervosa. We suggested fetally CNS masculinized females become nauseated at estrogen doses similar to males, lower than normal females & within the range normally produced by the ovaries (Gustavson, C.R. & Gustavson, J.C., Soc. Neurosci. meetings, 1986, & 1987; Gustavson, C.R., Gustavson, J.C., Young, J.L., Pumariega, A.J. & Nicolaus, L.K. Neur. Cont. Of Reprod. Func.: Proc. of Fifth Gal. Neurosci. Symp., 1988). We suggested that DES exposure may produce results similar to the testosterone masculinization we investigated previously.

This presentation reports: 1) the results of adult conditioned taste aversion establishment using 3 estrogen doses in post-natally DES & testosterone exposed, & unexposed male & female rats (N=> 6 rats/group); 2) an analysis of data from 1711 DES fetally exposed & 919 unexposed human females showing a rate of 18.7/1000 cases of unexplained low body weight (Weight<=80% of expected for age, sex & height) in the exposed group compared to 3.3/1000 cases in the unexposed group; a 5.72 to 1 ratio

452.7

PUTATIVE AMINO ACID NEUROTRANSMITTERS AND SOMATOSTATIN DYNAMICS OF THE VENTROMEDIAL AND LATERAL HYPOTHALAMUS DURING MEAL FEEDING. S.J. Dodds*, M.C. Starke*, and R.C. Burgus* (SPON: D.F. Peterson). Dept. of Biochem., Oral Roberts Univ. Sch. of Med., Tulsa, OK 74171.

The Ventromedial (VMH) and Lateral Hypothalamus (LH) have classically been considered hunger and satiety centers. Neuroanatomical experiments were undertaken to elucidate neurotransmitter relationships within these nuclei.

The VMH and LH were dissected and assayed for free amino acid and somatostatin concentration in response to meal feeding regimen in 18- and 3-month old rats. Frozen transverse sections (20 µm) were taken from the hypothalamic area with every fifth section stained for histological verification. Sections were freeze-dried and nuclei surgically removed under 25X mag. The VMH and LH fragments were weighed and stored under vacuum at -20°C until assayed.

Five putative neurotransmitter amino acids--asp, glu, gly, GABA, and taurine--were assayed by pre-column orthophthalaldehyde derivitization and HPLC. Somatostatin was assayed by a radioimmune technique.

Somatostatin and taurine varied significantly between the feeding stages, suggesting taurine's role as a neurotransmitter releasing the peptide hormone somatostatin. Taurine levels increased with initiation of a meal in the VMH and decreased in the LH. Linear patterns continued to the end of the meal ($p < .001$). Similar relationships were seen with somatostatin; the correlation was significant ($p < .01$). Other trends were seen with GABA, gly and glu.

452.8

LONG TERM STABILITY OF FEEDING BEHAVIOR IN RATS. R. Bauman and R. Pastel (SPON: R. Wylie). Dept. of Medical Neurosciences, Walter Reed Army Institute of Research, Washington, DC 20307.

Although previous research has documented the 24 hour patterns of feeding in rats, little if any data are available showing the long term stability of those patterns. Since the day to day stability of feeding patterns was considered a prerequisite to subsequent drug studies, the food intake of six rats was studied for 18 days. A 12 hour light/dark cycle was maintained in each rat's chamber. Rats could feed ad lib by pressing a lever once for each 45 mg food pellet. A meal was defined as a bout of eating not interrupted by a pause longer than 10 mins.

Food intake was much greater during the dark period. Hourly intake during the light was low and variable until the two hours preceding light offset, at which time food intake began increasing. Intake increased greatly at dark onset and peaked 3-4 hours into the dark period. The number (12 vs 3) and size (43 vs 32 pellets) of meals were significantly larger during the dark than during the light. However, the duration of a meal was not significantly larger during the dark period. Thus, the rate of eating (pellets/min) was greater during the dark, since meal durations were similar, but meal size was greater during the dark. Since the light/dark differences in meal structure and hourly food intake did not vary significantly over the 18 day period, the data strongly suggest that 24 hour patterns of food intake are very stable.

452.9

EFFECT OF FOOD TEXTURE ON TONGUE MOVEMENT DURING ANTICIPATION, CHEWING AND SWALLOWING IN CHILDREN SIX MONTHS TO TWO YEARS OF AGE.

E.G. Gisel. School of Physical and Occupational Therapy, McGill University, Montreal, Quebec, H3G 1Y5.

Tongue mobility changes rapidly in the human infant during weaning. The antero-posterior movements of suckling which are activated in concert with the mandible, hyoid and lower lip give way to more independent movements in a lateral direction, for placement of food between the teeth. Specific tongue movements in response to different textures of food (puree, viscous, solid) are not yet defined.

Our study describes the effect of food textures on tongue movement during anticipation, chewing and swallowing in 143 normal children from 6 months to 2 years of age. Taste was controlled by using sweet flavors. Children anticipate all three textures by holding the jaw and tongue quiet as food approaches the mouth. When initiating chewing, younger children (6-12 months) respond to puree by suckling, whereas older children (18-24 months) collect the food bolus in preparation for swallowing. The solid and viscous textures are chewed vigourously in a "munching" pattern where the tongue stays in mid-line in children up to 10 months, but with increasing lateral movements between 12 and 24 months. Three categories of swallow patterns were observed for all textures. Children either pressed their lips together to initiate the swallow, drew in the lower lip, or projected the tongue onto the lower lip with puree or the viscous texture. During anticipation, chewing and swallowing pureed textures elicit more immature patterns than solid or viscous textures.

Supported by NHRDP # 6605-2430.

452.10

IN VITRO AUTORADIOGRAPHIC MAPPING OF A PUTATIVE "ANORECTIC" BINDING SITE WITH [^{3}H] MAZINDOL IN RAT BRAIN. G. P. Vincent and B. E. Levin. Institute Animal Behav., Rutgers University, Newark, NJ 07102, Neurology Service, VA Medical Center, East Orange, NJ 07019.

Low affinity (µM) binding to hypothalamus of the anorectic agent mazindol (MAZ) correlates highly with its anorectic potency (Angel, et al., Brain Res. Bull. 17:873, 1986). These sites were mapped using quantitative in vitro receptor autoradiography. Coronal sections (20µm) were incubated with 10nM [^{3}H] MAZ (SA=15.8Ci/mmole) in 50mM Tris/5mM KCl, pH 7.9, at 4°C for 40min. Non-specific binding was assessed with 1mM MAZ. The distribution of these sites was different from that of the high affinity, sodium-dependent binding of [^{3}H] MAZ to catecholamine reuptake sites which were highly localized in the striatum. Areas of high specific [^{3}H] MAZ binding (>3.0pmol/mg protein; 53-69% of total binding) to the low affinity site were found in the dorsal raphe n., locus coeruleus, med. eminence, olf. tub., pineal gland. Moderate binding (1.6-3.0pmol/mg) was in the paraventricular, arcuate & ventromed. hypothalamic n., med. preoptic area, n. accumbens, amygdala, gustatory cortex, bed n. stria terminalis, periaquaductal gray, n. tr. solitarius. Low levels (<1.6pmol/mg) were in the caudate-putamen, dorsomed. n. & lateral hypothalamus, substantia nigra. This binding may thus represent a distinct class of sites related to the anorectic action of MAZ.

452.11

ACTIVITY-BASED-ANOREXIA AS A FUNCTION OF ACTIVITY-STRESS ULCERS. L.E. Doerries*, P.F. Aravich, E.Z. Stanley,* and J. Ang* (SPON: D.B.West). Dept. of Psychology, Christopher Newport College, Newport News, VA 23606; Dept. Anatomy & Cell Biology and Dept. Internal Medicine, Eastern Virginia Medical School, Norfolk, VA 23510.

Paradigms for studying activity-based anorexia (ABA) and activity-stress gastric ulcers (ASUs) involve concurrent introduction of restricted feeding and opportunity for exercise. While ABA is related to the simultaneous introduction of diet and exercise, it is possible that ASUs contribute to the phenomenon. Also, there is evidence suggesting that susceptibility to both ABA and ASUs may be gender specific. This study tests the hypothesis that ABA is unrelated to the formation of ASUs in body-weight matched, pubescent male and female rats. A 25% weight-loss criterion was used to define ABA. The rats (41-43 days old) were fed for 90 min. and allowed 22.5 hours access to an activity wheel. Although female rats ate less and ran more than males, both males and females reached the weight-loss criterion in the same number (6.13) of days. Sacrificed at 75% of their original body weight, no animal showed gross evidence of lesions or ulcers. We conclude that at this weight loss criterion ABA is not gender specific and is not attributable to the presence of gastric lesions or ulcers.

452.12

STUDIES ON THE INGESTIVE BEHAVIORS INDUCED BY INJECTIONS OF MUSCIMOL INTO THE MEDIAN RAPHE NUCLEUS OF THE RAT. D. Wirtshafter, T.R. Stratford, M.A. Klitenick, R. Trifunovic and J.C. Krebs. Dept. Psychol., Univ. Ill. at Chicago, Chicago, IL 60680.

We have demonstrated in the past that robust increases in food and water intake can be produced in nondeprived rats by injections of the GABA agonist muscimol into the median raphe nucleus (MR). Much larger effects are seen following injections into the MR than after injections into surrounding structures. In the present studies we have attempted to provide more detailed information as to the mechanisms underlying these effects.

Injections of muscimol into the MR induced wood gnawing and paper shreding, as well as feeding and drinking, suggesting that these injections may elicit both ingestive and noningestive oral behaviors. Intra-MR muscimol injections increased the rates of food reinforced lever pressing by nondeprived rats; in contrast, pressing of a nonreinforced lever was much less affected. This finding suggests that intra-MR muscimol injections render food reinforcing to nondeprived rats. Muscimol injections led to a mild hyperglycemia, but failed to alter plasma levels of insulin, ketone bodies or electrolytes. Like normal feeding, muscimol induced feeding was abolished in rats with large lateral hypothalamic lesions. Finally, haloperidol, amphetamine and fenfluramine were equipotent in suppressing deprivation and muscimol-induced feeding.

452.12

APOMORPHINE-INDUCED FLAVOR-DRUG ASSOCIATIONS: A DOSE-RESPONSE ANALYSIS BY THE TASTE REACTIVITY TEST (TRT) AND THE CONDITIONED TASTE AVOIDANCE TEST (CTA). L.A. Parker. Dept. of Psychology, Wilfred Laurier University, Waterloo, Ontario, N21 3C5.

Apomorphine is a positively reinforcing drug at low to moderate doses, but appears to lose its reinforcing properties at higher doses. Across a range of doses (0.5 - 15 mg/kg, intraperitoneally), apomorphine produced a CTA over 5 conditioning/testing trials which was not dose-dependent. The rejection TRT responses of chin rubbing and gaping, however, were only conditioned at the highest dose of apomorphine (15 mg/kg). Our results support the contention that CTAs produced by positively reinforcing drugs are not accompanied by a palatability shift.

452.14

c-fos INDUCTION IN NUCLEUS TRACTUS SOLITARIUS AND IN THE DORSOMEDIAL NUCLEUS OF THE HYPOTHALAMUS DURING FEEDING IN RATS. M.F. Gonzalez, S.M. Sagar, and F.R. Sharp, Depts. of Neurology and Physiology, UCSF and VA Medical Center, San Francisco, California 94121.

The CNS pathways involved in the production of satiety are still poorly understood. In order to test whether starvation or feeding would induce Fos, the protein product of the c-*fos* proto-oncogene, the following experiments were performed. Adult Sprague-Dawley rats were starved for 1,2,3 or 6 days. Half of the subjects were allowed to feed and sacrificed 4 hours later. Brains of starved subjects and starved subjects allowed to feed were processed for Fos immunocytochemistry using a polyclonal antiserum to a middle peptide of Fos raised in our laboratory. Fos was markedly induced throughout the nucleus tractus solitarius (NTS) and nucleus ambiguus (NA) in the medulla, and in the dorsomedial nucleus of the hypothalamus (DMH) of the subjects allowed to feed compared to the starved controls and compared to ad libitum controls. Fos induction in DMH but not NTS was increased with longer periods of starvation. It is proposed that at least one satiety signal arises in the GI tract, travels via the vagus to NTS, and from there to the DMH. Because of the proposed role of c-*fos* in regulating other genes, it is possible that neural signals and responses to feeding are programmed by Fos.

452.15

PERIFORNICAL HYPOTHALAMIC MICROINJECTIONS OF AMPHETAMINE AND PHENYLPROPANOLAMINE: EFFECTS ON FEEDING IN THE RAT. P.J. Wellman, R. Cockroft* and S. Keller*. Dept. of Psychology, Texas A&M University, College Station, TX 77843.

Amphetamine anorexia results from activation of dopaminergic and/or beta-adrenergic cells within the perifornical hypothalamus (PFH) but the locus of anorexia induced by the amphetamine analogue phenylpropanolamine (PPA) is as yet unidentified. In the present experiment, adult male rats were allowed to consume a palatable sweetened-mash diet for a 30 minute period after 14 hours deprivation of food, but not water. Microinjections (0.5 ul/40 sec) of either 20, 40, 80, 160 or 320 nm PPA or 100 nm d-amphetamine (calculated as the base) into the perifornical hypothalamus were given 5 minutes prior to a feeding trial with vehicle trials interspersed between drug trials. Amphetamine significantly lengthened latency to feed and suppressed feeding. PPA was without significant effect on feeding at any dose. These data support the contention that although amphetamine and PPA induce superficially similar anorexia, the mechanisms by which these drugs act on feeding are independent.

452.16

CIRCADIAN FLUCTUATIONS IN TASTE PREFERENCES IN RATS. D. Dracos and F.W. Flynn. Dept. of Psychology and Neuroscience Program, Univ. of Wyoming, Laramie, 82071.

Day/night fluctuations in internal state are thought to mediate the periodicity in ingestive behavior in animals. Since experimental manipulations of metabolism and water/electrolyte balance modify taste processing, the effects of circadian changes in internal state on taste preference were measured. Rats were entrained to a 12 h light/dark cycle. Two bottles, one containing water and the other the taste stimulus (sucrose - 0.01 M, 0.03 M, 0.3 M, 1.0 M; quinine HCl - 0.00003 M, 0.0003 M, 0.003 M, 0.03 M; HCl - 0.001 M, 0.003 M, 0.01 M, 0.03 M; NaCl - 0.01 M, 0.03 M, 0.1 M, 0.3 M), were presented at the onset of either the light cycle (N=15) or dark cycle (N=14). Intake was measured 12 h later and preference computed. During the dark period, the preference for 0.03 M. sucrose (87 $\pm$ 2%) and 0.1 M NaCl (85 $\pm$ 3%) were significantly greater than that recorded during the light period (55 $\pm$ 6% and 66 $\pm$ 6%, respectively), p's $<.05$. Also, during the dark phase, the rats' preference for QHCl concentrations was consistently lower than that of the light phase, p's $<.05$. There were no significant group differences in HCl preference. The results demonstrate that the rats' preferences and avoidances of taste stimuli are influenced by the light cycle.

(Supported by NIH grant R01 NS24879 to F.W.F.)

453.1

1-METHYL-4-PHENYLPYRIDINIUM ION (MPP+)- INDUCED MOTOR DEFICITS IN THE RAT. S.L. Walsh, P. McCarty* and G.C. Wagner. Psychology Department, Rutgers University, New Brunswick, NJ 08903

MPP+ is the active metabolite of MPTP, a potent parkinsonian neurotoxin. MPTP administration produces a parkinsonian syndrome in primates, however rodents are comparatively resistant to the toxic effects of this compound. To assess motor deficits following administration of intrastriatal MPP+ (20ug/side), Sprague-Dawley male rats were tested using two behavioral procedures; the rotarod for balance and gait and the force lever task for fine motor control.

Following eight daily sessions on the rotarod, it was observed that control rats were able to maintain balance on the rotating rod (12 rpm) for an average of 130 sec of a possible 180 sec, whereas the lesioned rats were significantly impaired, averaging only 84 sec. In the force lever task, control rats (trained to exert between 10 and 15 g of paw pressure for 1 second in order to procure a water reinforcement) required an average of 4 band entrances/reinforcer, whereas lesioned rats were significantly impaired, requiring an average of 8 band entrances. This indicates that, although lesioned rats obtained a comparable number of reinforcers as the controls, they performed less efficiently. In addition, using drug challenge, it was observed that lesioned rats were sensitized to the acute effects of apomorphine on the rotarod task and were more sensitive to the acute effects of oxotremorine on the force lever task as measured by estimated ED-50 doses. These observations are evaluated in light of the magnitude of the MPP+-induced neurochemical lesion of the striatum and the degree of impairment observed.

453.2

HALOPERIDOL-INDUCED MOVEMENT DISORDERS IN YOUNG AND AGED RATS. S.K. Johnson*, J. Lee*, H. Fisher* and G.C. Wagner. Depts. of Psychology and Nutrition, Rutgers Univ. New Brunswick, NJ 08903

Movement disorders were studied in groups of young (60-day old) and aged (about one year) male rats for a period of three months following acute exposure to high doses (25 mg/kg) of haloperidol. It was observed that 2 injections of haloperidol spaced 3 weeks apart resulted in an increase in chewing behavior, head twitching and side-to-side movements beginning one week following the first injection and continuing through the three month observation period. Aged rats exhibited an increase in the intensity and frequency of these behaviors as compared to younger rats for both haloperidol and vehicle treatments. In addition, the high dose treatment with haloperidol produced a long-lasting disruption of rotorod performance, lasting 140 h in young rats and 300 h in aged rats. These observations are interpreted in light of haloperidol-induced changes in dopamine turnover and/or dopamine receptor proliferation, and in relation to the etiology of tardive dyskinesia.

453.3

Neural Mechanisms for Plan Execution and Plan Selection

David Hestenes, Arizona State University
Daniel S. Levine, University of Texas at Arlington

Some emerging data are suggesting functions for pathways from the midbrain with monoamine (norepinephrine, dopamine, and serotonin) neurotransmitters. One pathway, involving the basal ganglia, appears to be important in the selection of motor plans. Another pathway, involving the limbic system, appears to be involved in ensuring that plans once selected are actually executed. Different control functions are suggested for each of the transmitters in pattern matching. The prefrontal area of the cerebral cortex appears to be engaged in integration of these two circuits. Such characteristic motor disorders as Parkinson's disease, La Tourette's syndrome, and obsessive-compulsive disorder (which has both motor and ideational components) can be understood as breakdowns in one or another circuit.

453.4

ACUTE AND CHRONIC EFFECTS OF MONOAMINE UPTAKE BLOCKADE ON PGO WAVES IN THE CAT. R.J. Ross, W.A. Ball*, D.R. Levitt, P.J. Gresch* and A.R. Morrison. VA Med. Center and Depts. of Psychiatry and Animal Biology, Univ. of Penna. Schools of Med. and Vet. Med., Phila., PA 19104.

Spontaneous ponto-geniculo-occipital (PGO) waves are recorded in the lateral geniculate body (LGB) of the cat during paradoxical sleep (PS). They may signal alerting and are likely similar to waves that accompany orienting to intense or novel stimuli in waking (W). Depletion of norepinephrine (NE) or serotonin (5HT) results in PGO waves during W, and such waves are inhibited by drugs that block monoamine uptake and that have antidepressant effects in humans. We studied changes in spontaneous PGO waves after acute and chronic desipramine (DMI) and sertraline (SER), which are relatively specific in blocking NE and 5HT uptake, respectively. Nine cats were implanted with EEG, EOG, EMG, and LGB electrodes. Initial placebo days were compared with the first day (acute) of p.o. drug (0.75 mg/kg) and with days (chronic) that followed 2.5 weeks of p.o. drug (2.0 mg/kg q.d.). PGO rate (per min.) during PS was not significantly decreased by acute DMI (51.4 vs. 50.7, $p>.05$) or acute SER (59.5 vs. 56.4, $p>.05$). PGO rate dropped after chronic DMI (56.4 vs. 38.7, $p<.05$) and chronic SER (56.1 vs. 48.8, $p<.05$). Thus, chronic NE or 5HT uptake blockade suppresses PGO waves. The mechanisms may relate to antidepressant effects, since depression has features of hyperarousal (Gold, P.W. et al., N. Engl. J. Med., 319:413, 1988). Supported by VA Med. Res. Serv.

453.5

ELECTROPHYSIOLOGICAL INTERACTIONS BETWEEN SIGMA AND DOPAMINE RECEPTOR LIGANDS ON MIDBRAIN DOPAMINE NEURONS. G. F. Steinfels, B. Wolfson, L. Cook, S. W. Tam. DuPont Co., Med. Prod. Dept., Wilmington, DE 19880-0400, U.S.A.

Autoradiographic studies which have demonstrated the presence of sigma receptors on substantia nigra dopamine (DA) neurons have prompted the speculation that sigma receptors may have a modulatory role in DA neurotransmission. Support for this hypothesis comes from in vivo studies demonstrating that sigma receptor agonists [(+)-3-PPP, (+)-pentazocine, DTG] decrease the DA single unit activity, and the sigma receptor antagonist BMY-14802 increases DA single unit activity. This study evaluated the interactions between haloperidol and the sigma antagonist BMY-14802. Haloperidol, up to doses of 1 mg/kg, i.v., does not produce its typical increase of substantia nigra DA unit activity when pretreated with 1 mg/kg BMY-14802. This suggests a role of the sigma receptor in the nigro-striatal feedback loop which is thought to be responsible for the DA unit rate increases produced by haloperidol. The BMY-14802 dose response curve for producing DA unit rate increases was minimally affected following pretreatment with haloperidol. Since BMY-14802 does not bind to the DA agonist or antagonist receptor sites, we hypothesize that the sigma receptor-induced rate increases are mediated at the DA cell body.

453.6

SINGLE-UNIT ACTIVITY IN THE NEOSTRIATUM OF FREELY MOVING RATS: EFFECTS OF CORTICAL LESIONS ON THE BEHAVIORAL AND NEURONAL RESPONSES TO AMPHETAMINE AND NEUROLEPTICS. J.T. Tschanz*, J.L. Haracz, K.E. Griffith*, G.V. Rebec (SPON: R.M. Wightman). Prog. Neural Sci., Dept. Psychology, Indiana University, Bloomington, IN 47405.

Single neostriatal neurons in freely moving rats show either increases or decreases in firing rate in response to amphetamine (AMP), with increases predominating among cells showing enhanced activity during movements such as locomotion, rearing, or head movements (Haracz et al., Soc. Neurosci. Abstr. 14:664, 1988). These neuronal changes may reflect a direct action of AMP in the neostriatum or may be secondary to an action of the drug at some remote site. The neostriatum receives substantial input from cerebral cortex, which is known to respond to AMP. To study the role of the cerebral cortex in the response of neostriatal neurons to AMP, rats sustained bilateral ablations of frontal and sensorimotor cortex at least one week prior to single-unit recording. All animals were challenged with 1.0 mg/kg D-AMP during simultaneous videotaping of behavior and electrophysiological recording. As previously found in intact rats (Haracz et al., ibid), AMP-induced excitations and inhibitions predominated in motor- and nonmotor-related cells, respectively. Thus AMP activated 9 of 10 motor-related cells and inhibited all 4 nonmotor-related cells. These results differ from the predominantly inhibitory effects of AMP seen in multiple-unit recordings (Warenycia et al., Neuropharmacol., 26:1107, 1987). Despite an increased behavioral response to AMP in lesioned rats ($p < 0.05$), the AMP-induced enhancement of movement-related neuronal activity was significantly reduced as compared to sham-lesioned ($p < 0.05$) and intact controls ($p < 0.0005$). This reduction may be due in part to ceiling effects since both pre- and post-AMP movement-related unit activity were enhanced in lateral and central neostriatum of lesioned animals. Thirty min after AMP, animals were challenged with either 1.0 mg/kg haloperidol (HAL) or 10.0 mg/kg clozapine (CLZ). As reported previously (Haracz et al., op cit), HAL produced suppressions in all cells activated by AMP, whereas CLZ produced both excitations and inhibitions. In summary, a major portion of the AMP-induced enhancement of motor-related activity depends on intact corticostriatal projections. [Supported by USPHS grant DA 02451.]

453.7

SINGLE-UNIT ACTIVITY IN MEDIAL NEOSTRIATUM AND NUCLEUS ACCUMBENS: EFFECTS OF AMPHETAMINE, HALOPERIDOL, AND CLOZAPINE IN FREELY MOVING RATS. J.L. Haracz, J.T. Tschanz*, J.M. White*, Z.R. Wang*, D.W. Miller*, and G.V. Rebec. Prog. Neural Science, Dept. Psychology, Indiana Univ., Bloomington, IN 47405.

We previously reported that haloperidol (HAL) and clozapine (CLZ) differentially affected amphetamine (AMP)-induced changes in behavior and single-unit activity in lateral neostriatum of freely moving rats (Haracz et al., Soc. Neurosci. Abstr. 14:664, 1988). We now extend these observations to medial neostriatum and nucleus accumbens in rats subjected to simultaneous videotaping of behavior and recording of single-unit activity. To date, 17 neurons exhibited firing rates of 1.92±0.78 spikes/sec during quiet rest, 14 of which were activated during motor activity, such as locomotion (n=9) or head movements (n=5). Many neurons altered their activity in relation to the probing of specific body regions. D-AMP (1.0 mg/kg) was administered 30 min before either HAL (1.0 mg/kg) or CLZ (10.0 mg/kg). AMP-induced stereotyped behaviors developed in correspondence with increases (n=13) or decreases (n=4) in neuronal activity, with increases predominating among motor-related neurons. HAL inhibited behavior while suppressing the activity of all 13 cells tested. Compared to lateral neostriatal neurons (Haracz et al., ibid), cells in medial neostriatum or nucleus accumbens were suppressed significantly less by HAL ($p < 0.025$). Paradoxically, 3 cells were inhibited by both AMP and HAL. In contrast to HAL, CLZ produced both increases and decreases in activity. These findings differ from results obtained from immobilized rats, in which acutely administered neuroleptics either elevated or did not affect neuronal activity in neostriatum and nucleus accumbens (Rebec et al., Neuropharmacol., 19:281, 1980; Akaike et al., Life Sci., 32:2649, 1983; Le Douarin et al., Brain Res., 363:290, 1986). The regional variation in HAL effects and the differential effects of HAL and CLZ may underlie the different behavioral effects of these drugs. [Supported by USPHS grant DA 02451.]

453.8

A NEW COMPUTER PROGRAM FOR THE CONTROL OF IN VIVO BRAIN SELF STIMULATION: APPLICATION TO THE EFFECTS OF A NEW CLASS OF CENTRAL STIMULANTS. T. Kling-Petersen* and K. Svensson* (spon. J. Engel). Dept. of Pharmacology, Univ. of Göteborg, PO Box 33031, S-400 33 Göteborg, Sweden.

The in vivo brain self stimulation paradigm has been used for many years in order to investigate the biological substrates of reward or positive reinforcement. In general, central stimulants acting by the catecholamine neurotransmitters (e.g. d-amphetamine) or the opiod system (e.g. morphine) facilitate self-stimulation. Recently, our group presented a new class of central stimulants: "preferential dopamine autoreceptor antagonists" (K. Svensson et al., Naunyn-Schmiedeberg's Arch Pharmacol, 1986,334, 234-245). This class of compounds (exemplified by (+)-AJ76) produces mild stimulation over a wide dose-range. The degree of stimulation is however limited by the postsynaptic dopamine receptor blockade induced by large doses of the drug. It would be of great interest to have these types of compounds characterized in the self-stimulation paradigm.

A fully automated self stimulation method is currently being set up. This system will be based on standard sound-proof lever pressing boxes and physiological stimulators. The equipment will be controlled by an Apple MacIntosh™ IIx computer with 8 Mb primary memory and the National Instruments Lab VIEW program with a 12 bit analog/digital converting board. Our main objectives when setting up the method are: possibility to record at least 4 animals simultaneously, ability to vary the response rate of the different animals independently, to get total calculations at specified time intervals and continuously record them on disc.

The computer program will be presented and, if available, preliminary data on the new type of central stimulants as well as reference compounds.

453.9

DOPAMINE ANTAGONIST ACTIVITIES OF (+)-AJ 76. A.H.Tang, S.R.Franklin, C.S.Himes* and R.A.Code* CNS Research, The Upjohn Company, Kalamazoo, MI 49001

(+)-AJ 76 was reported by Svensson et al. (1986) to have a profile of activity suggestive of selective dopamine autoreceptor antagonism. This study compared (+)-AJ 76 to the specific D2 antagonist, sulpiride, in dopamine-related activities. Spontaneous locomotor activity of rats was significantly increased at low doses and reduced at higher doses by both compounds. (+)-AJ 76 is similar in potency to sulpiride. The locomotor depressant effect from a low dose (0.03 mg/kg) of apomorphine was partially reversed by both (+)-AJ 76 and sulpiride. Both compounds also antagonized yawning produced in rats by the selective D2 agonist, quinpirole (0.06 mg/kg). (+)-AJ 76 is slightly more potent than sulpiride. Hypothermia effect produced by quinpirole (1 mg/kg) in mice is completely antagonized by the pretreatment with either (+)-AJ 76 or sulpiride. On the other hand, stereotypic licking and chewing produced by a high dose (3 mg/kg) of apomorphine in rats was not antagonized by either compound even at a high dose of 100 mg/kg. The emetic effect of apomorphine (0.1 mg/kg) in dogs was effectively blocked by sulpiride (ED50=0.2 mg/kg). (+)-AJ 76 did not antagonize apomorphine in this test at doses up to 3 mg/kg. We conclude that (+)-AJ 76 is similar to sulpiride as a selective D2 antagonist. Both compounds preferentially antagonize dopamine at the autoreceptors.

453.10

FETAL NIGRAL DOPAMINE TISSUE TRANSPLANTS IN THE LATERAL STRIATUM OF RATS: EFFECTS ON LICKING. J.B. Richards, K.E. SABOL, and C.R. FREED. Univ. of Colo. Health Sci. Ctr., Denver, CO 80262.

The striatum is functionally heterogeneous in that anatomically discrete areas of the striatum control topographically different behaviors. Recently, Pisa & Schranz (Behav. Neurosci..102:429-440, 1988) using ibotenate-induced lesions, and Kelley et al. (Psycopharm..95:556-559, 1988) using micro-injections of d-amphetamine, have shown that the ventral lateral striatum is involved in mediation of tongue and mouth movements. We report the effects of placing fetal nigral dopamine tissue grafts in the striatum on the behavior of rats licking drops of water. The rats were first given unilateral 6-hydroxydopamine lesions and tested for amphetamine induced rotation to assure they had greater than 95% depletion. Fetal nigral transplants from 12 to 15 day old rat fetuses were then placed into the ventral lateral (n=7) and dorsal medial (n=5) striatum of the lesioned side. A control group (n=5) was lesioned but did not receive fetal grafts. Nine months after transplantation, the rats were exposed to a schedule of periodic water presentation in which they received a .025 ml drop of water every 30 sec over a 30 min period. There were no group differences in baseline licking behavior. When given 0.1 mg/kg d-amphetamine rats with lateral but not medial transplants licked more than controls.

453.11

BEHAVIORAL AND NEUROCHEMICAL EFFECTS OF ACUTE AND CHRONIC MICROINJECTIONS OF A K^+-CHANNEL BLOCKER INTO THE VENTRAL TEGMENTAL AREA OF RATS. J.D. Steketee and P.W. Kalivas. Dept. of VCAPP, Washington State University, Pullman, WA 99164-6520.

Apamin is an octadecapeptide which has been reported to block small conductance Ca^+-activated K^+-channels (SK). Shepard and Bunney (Brain Res. 463:380, 1988) have demonstrated that apamin increases dopamine (DA) neuron excitability in the substantia nigra of rat brain slice preparations. Increased activity in the mesolimbic dopamine system has been linked to increased motor activity. We investigated the effects of microinjections of apamin into the ventral tegmental area (VTA) of rats. Acute bilateral microinjections of apamin into the VTA led to a dose-related (0.1 - 3.0 pmoles/side) increase in motor activity. HPLC analysis of postmortem brain tissue obtained 30 min post-injection revealed a dose related increase in DA metabolism in both the VTA and the nucleus accumbens, an axonal terminal field of the VTA. Daily microinjections of apamin into the VTA appeared to initially lead to tolerance followed by an augmentation of motor activity one week after chronic treatment. These results support a role for SK channels in modulating DA stimulated motor activity.

453.12

RESTRAINT INDUCED ALTERATIONS IN INTRACRANIAL SELF-STIMULATION FROM THE NUCLEUS ACCUMBENS OF BALB/cByJ, C57BL/6J and DBA/2J MICE: EFFECTS OF DIAZEPAM. G. MacNeil, N. Gabora*, S. Collins* and R. Zacharko, Dept. Psychology, Carleton University, Ottawa, CANADA

Uncontrollable stressors induce disturbances in responding for electrical brain stimulation (ICSS) from both mesolimbic and mesocortical sites. Investigations in this laboratory focused on the effects of uncontrollable footshock on ICSS in both inbred and non-inbred mouse strains. In the present report the effects of restraint were evaluated on ICSS from the nucleus accumbens in the BALB/cByJ, C57BL/6J and DBA/2J strains. While protracted immobilization was associated with deficits in ICSS among BALB/cByJ and DBA/2J mice, such effects were absent in the C57BL/6J strain. These ICSS deficits were restricted to the anterior plane of the accumbens and prophylactic administration of diazepam produced an attenuation but not a complete reversal of the ICSS alterations. It is suggested that the anatomical/neurochemical integrity of the mesolimbic system in a rostral-caudal plane underlies the disparity in behavioral effects observed.

453.13

STRAIN DIFFERENCES IN RESPONDING FOR SELF STIMULATION FROM THE VENTRAL TEGMENTUM FOLLOWING ACUTE AND CHRONIC SHOCK. M. Kasian* and R.M. Zacharko, Dept. Psychology, Carleton University, Ottawa, Ontario K1S 5B6, Canada.

Acute and chronic footshock influenced self stimulation responding from the lateral aspects of the ventral tegmentum. However, the extent of the disruption induced by acute shock and the course of the adaptation associated with a chronic stressor varied across strains of mice. In C57BL/6J and CD-1 mice a single shock session provoked a persistent disruption of ICSS performance. Responding recovered to baseline rates within 5 shock sessions, and in C57BL/6J mice responding exceeded basal rates after 10 sessions. In contrast, in DBA/2J mice the decline of responding was not evident until 10 shock sessions were applied, and recovery of responding was evident after 15 sessions. Finally, in BALB/cByJ mice increased rates of responding were evident regardless of the number of stressor sessions applied. It is suggested that analyses of stressor related behavioral disturbances should consider genetic differences in the impact of acute stressors and in the adaptation associated with chronic stressor regimens.

453.14

Ontogeny of dopamine-mediated behavior and D1 receptors in three rat strains. Frances Petracca*, Jaime Diaz, and Alan S. Unis. Departments of Psychiatry and Psychology University of Washington, Seattle, Washington 98195

The F344 rat is a proposed model of Attention Deficit-Hyperactivity Disorder (ADHD) because of its increased locomotor behavior compared to the Buffalo (BUF) rat. Our preliminary observations regarding the ontogeny of this behavior are reported here. D-1 receptors, which mature functionally before D-2 receptors, were also examined. Ontogeny of developmental milestones, reflexes, and open field behavior were studied in pups from these two strains and the Sprague-Dawley (SD).

3H-SCH23390 autoradiography revealed anatomical localization to cortical regions in Day 4 pups which was eliminated by Day 21. Striatal binding was consistent from Day 4 to 21. Significant strain-related differences in indices of maturation and behavior are shown below:

	F344	SD	BUF
Weight (g)	32.5± 3.1	55.5 ± 4.7	38.4± 5.7
Eye opening (day)	17.7± .5	15.3 ± .5	16.1± .3
Free fall righting	16.5± 1.1	15.9 ± 1.0	17.8± 1.2
Open Field rears	20.2±12.7	22.6 ± 11.5	8.1± 8.2

These studies may support the use of the F344 rat as a model of ADHD.

NEUROETHOLOGY III

454.1

The One-Dimensional Protein Profiles of Gymnotiform and Mormyriform Electric Organ Homogenates are Similar. J. M. Patterson* and H. H. Zakon, The University of Texas at Austin, Dept. of Zoology, Austin, Texas 78712. The two orders of electric fish, the gymnotiforms of South America, and the Mormyriforms of Africa, possess distinct phylogenetic histories, distinct electric organ discharge patterns and waveforms, and show large differences in electric organ morphology. However, both groups have electrocyte cells which are embryologically derived from muscle cells. To determine what differences might exist in the proteins present in the Mormyrid and gymnotiform electric organ, and to compare the protein composition of both electrocyte cells and skeletal muscle cells from various species, one-dimensional gel electrophoresis was used.

Electric organ tissue from six species and muscle tissue from three species of electric fish were analyzed. Electric organs from four gymnotiform species (*Sternopygus* , Sach's and Main organ from *Electrophorus* , *Hypopomus* , and *Eigenmannia humboldtii*) and two Mormyrid species (*Gnathonemus petersii* and *Brienomyrus niger*) were compared. Muscle tissue from one gymnotiform (*Sternopygus*) and from the two Mormyrid species was obtained from a dorsal portion of the fish.

Segments of electric organ or muscle were cleaned of skin and immediately frozen in liquid nitrogen. The tissue was then homogenized in a SDS/BME sample buffer, placed in a 100° C water bath for 3 min., centrifuged at 10,000xG, and frozen at -20° C. Gels were run at constant voltage (125 v. for 7-8 hr.), stained with Coomassie Blue, destained, and photographed. Densitometry was performed on several gels for a more quantitative evaluation of protein composition.

Comparisons between the muscle cell and electric organ proteins show many shared constituent proteins, indicative of their common origins. However, several proteins are found in abundance in electric organ , yet are not present or present in low abundance in muscle. Of these non-muscle proteins, analysis shows marked similarities in the protein profiles from all the species of electric fish studied. This finding indicates that despite independent evolutionary histories, Mormyrid and gymnotiform fish have developed similar biochemical components for electric organ function.

454.2

Synaptic Plasticity in the Cerebellar Parallel Fiber Projection to the Electrosensory Lateral Line Lobe of Gymnotiform Fish. R.W. Turner* and L. Maler (SPON: W. Hendelman) Dept. of Anatomy, University of Ottawa, Ontario, Canada K1H 8M5

Pyramidal cells (PC) of the electrosensory lateral line lobe (ELL) of the weakly electric fish *Apteronotus leptorhynchus* receive direct afferent input from peripheral electroreceptors involved in the behaviour of electrolocation. PC apical dendrites in the ELL molecular layer receive a glutaminergic parallel fiber input from granule cells of the caudal cerebellar lobe that has been shown to regulate PC temporal and spatial receptive field properties (Bastian,86). As the molecular layer exhibits high densities of NMDA and Kainate receptors (Maler & Monoghan,89) frequently involved in plasticity of synaptic transmission, we used an *in vitro* slice preparation to examine PC responsiveness to parallel fiber stimulation.

Stimulation of the ELL molecular layer evoked a fast positive-negative and a slow negative-going field potential in the PC mid-dendritic region. Intracellular recordings and perfusion of low Ca^{+2} medium identify these potentials as a fiber volley and EPSP characteristic of that described for parallel fiber input in cerebellar cortex and dorsal cochlear nucleus. Low frequency stimulation (5-20 Hz) evoked a pronounced frequency potentiation of the dendritic field negativity that recruited PC discharge. High frequency tetanic stimulation (90-200 Hz, 15 pulses; 7X) evoked a long-term potentiation of the dendritic negativity (83 +/- 23% above control) that persisted for a minimum of two hours. One slice demonstrated post-tetanic potentiation (19 % above control) for 30 min while 3 slices showed no effect. Tetanic stimulation in low Ca^{+2} medium had no effect or evoked a depression of the parallel fiber volley for 20-30 min. The role of excitatory amino acid receptors in these forms of synaptic plasticity are under investigation.

454.3

DISTRIBUTION OF GLUTAMATE RECEPTORS IN THE ELECTROSENSORY SYSTEM OF GYMNOTIFORM FISH. L. Maler and D. Monoghan, University of Ottawa, University of California at Irvine.

Electroreceptor afferents of gymnotiform fish (Apteronotus leptorhynchus) project to the deep neuropil layer of a laminated hindbrain structure, the electrosensory lateral line lobe (ELL). The ELL projects to the nucleus praeeminentialis dorsalis (Pd) which has feedback projections to the ELL, both directly to the ventral molecular layer of the ELL, and indirectly (via the caudal cerebellar lobe LC) to the dorsal molecular layer of the ELL. The circuitry of the ELL has been analysed in detail and correlated with the spatial and temporal receptive field properties of the ELL pyramidal cells (PC). Bastian (1986) has demonstrated that the cerebellar feedback projection to ELL can modulate the PC receptive fields.

We examined the distribution of glutamate receptor subtypes (AMPA, Kainate and NMDA) in the ELL as well as in the midbrain. In the ELL AMPA receptors are found at low levels in the deep neuropil layer as well as throughout the molecular layer; AMPA receptors are also found at low levels in the molecular layer of the related caudal lobe, but are very dense in the cerebellar molecular layer. Kainate receptors are completely confined to the dorsal and cerebellar molecular layer, where they are very dense. NMDA receptors are present at high density in the molecular layer but only at low density in the deep neuropil, suggesting plasticity of the feedback loop.

454.4

MODULATIONS OF THE ELECTRIC-ORGAN PACEMAKER NUCLEUS OF GYMNOTIFORM ELECTRIC FISH BY PHARMACOLOGICALLY DISTINGUISHABLE PATHWAYS. M. Kawasaki, C.H. KELLER, W. Heiligenberg. UCSD, La Jolla CA 92093.

Both pulse and wave species of gymnotiform electric fish are capable of accelerating and decelerating the firing rate of their electric organ discharges (EODs) during social interactions. Modulatory inputs from the prepacemaker nucleus (PPN) to the medullary pacemaker, which drives the electric organ with a regular rhythm, are responsible for these frequency modulations. Physiological recordings from PPN neurons, stimulation of the PPN, and application of pharmacological blockers to the pacemaker nucleus have revealed a difference in the mode of the modulation between wave and pulse species. In a wave species, Eigenmannia lineata, the increase and the decrease of a tonic excitatory input to the pacemaker appears to be responsible for the acceleration and the deceleration of the EOD frequency, respectively. In a pulse species, Hypopomus brevirostris, on the other hand, direct GABA-ergic inhibition appears to decelerate the pacemaker, while glutaminergic excitation causes acceleration.

454.5

INTRACELLULAR LABELLING OF PHYSIOLOGICALLY DEFINED CELLS WITHIN A DIENCEPHALIC SENSORY-MOTOR INTERFACE OF WEAKLY ELECTRIC KNIFEFISH C.H. Keller, M. Kawasaki and W. Heiligenberg. UCSD, La Jolla CA 92093

The diencephalic complex of Eigenmannia's nucleus electrosensorius (nE) comprises a number of finely tuned neuronal filters for specific sensory-motor tasks. The nE can be subdivided into at least four distinct areas; three which receive electrosensory efferents of the torus semicircularis: nE↑ controls rises of the electric organ discharge (EOD) frequency, nE↓ controls falls of the EOD frequency, nEb contains neurons responsive to stimuli jamming the fish's EOD; a fourth area, nEar, receives acousticolateral but not electrosensory toral input. Intracellular labelling of cells within each subdivision demonstrates intranuclear patterns of connectivity and cell-specific efferent targets. We present data on a number of different cell types that are responsive to various electrosensory stimuli: e.g. ongoing jamming stimuli, rapid modulations in jamming stimuli similar to courtship signals, and changes in orientation of the jamming stimulus. Some neurons responsive to jamming stimuli project to the vicinity of electromotor control areas of the diencephalon and likely contribute to the jamming avoidance response. Also, cell-types that may provide information about socially relevant stimuli project to hypothalamic targets and may thereby modulate endocrine or motivational systems of the CNS.

454.6

ULTRASTRUCTURAL STUDIES ON THE SYNAPTIC ORGANIZATION OF THE PREPACEMAKER NUCLEUS IN WEAKLY ELECTRIC KNIFEFISH. W. Heiligenberg, G.K.H. Zupanc and L. Maler. UCSD, La Jolla, CA 92093; Univ. Ottawa, Ottawa, Ontario K1H 8M5.

Large multipolar neurons of the diencephalic prepacemaker nucleus (PPn) of knifefish (*Eigenmannia*) have been shown to control a communicatory behavior, namely abrupt frequency modulations ('chirps') of the otherwise very constant electric organ discharges. By retrograde labelling of the multipolar neurons with HRP we have investigated the synaptic organization of the PPn.

We found two classes, each with two subtypes, of chemical synapses contacting PPn neurons: (1a) Asymmetric synapse; only agranular, predominantly round vesicles (Ø 30-45 nm. (1b) 88-98 % agranular small vesicles as in 1a; otherwise, large (Ø 50-100 nm) dense-core vesicles. (2a) Symmetric synapse; small agranular pleiomorphic vesicles. (2b) 96-99 % small agranular pleiomorphic vesicles as in 2b; otherwise, large (Ø 50-70 nm) dense core vesicles. The synaptic cleft is wider in class 1 than in class 2 synapses. In addition to these chemical synapses we also identified dendro-dendritic gap junctions.

Electrotonic junctions might be responsible for a joint depolarization of PPn neurons during chirping. The chemical synapses are likely to mediate excitatory (class 1) as well as inhibitory input (class 2), each of which may be modulated by the release of the content of the dense-core vesicles (b-subtypes).

454.7

SOMATOSTATIN-LIKE IMMUNOREACTIVITY IN THE PREPACEMAKER NUCLEUS OF ADULT KNIFEFISH CHANGES WITH SEXUAL MATURITY. G.K.H. Zupanc, L. Maler and W. Heiligenberg. UCSD, La Jolla, CA 92093; Univ. Ottawa, Ottawa, Ontario K1H 8M5.

Knifefish (*Eigenmannia*; Gymnotiformes, Teleostei) can modulate their otherwise constant electric organ discharges by abrupt frequency modulations ('chirps'), a pattern which occurs almost exclusively during the tropical rainy season when these animals reproduce. Chirps are controlled by a subnucleus of the diencephalic prepacemaker nucleus, the PPn-C. The morphology of PPn-C neurons undergoes sexual maturity-dependent changes which might subserve the seasonal modifications in chirping behavior (Zupanc, G.K.H. & Heiligenberg, W., J. Neurosci., in press). In this study we investigated changes in somatostatin-like immunoreactivity (S-IR) in the PPn-C of mature and immature females.

We found three S-IR structures in the PPn-C region. While two were clearly expressed in both groups, the third pattern, a diagonal stripe (dS), was less pronounced in mature animals. The dS shows partial overlapping with dendrites of the ventral territory of PPn-C dendrites.

Somatostatin may act as a neuromodulator and thus cause reversible physiological changes in excitability of PPn-C neurons, or it could act in a hormone-like manner and lead to long-term morphological modifications as they occur during seasonal sexual maturation.

454.8

SEX DIFFERENCES IN THE ACTIVE SPACE FOR ELECTRIC SIGNALLING IN A SEXUALLY DIMORPHIC GYMNOTIFORM, *HYPOPOMUS PINNICAUDATUS* . C.D. HOPKINS[1], N. C. COMFORT[1], AND J. BASTIAN[2]. Section of Neurobiology and Behavior; [1] Cornell University; Ithaca, NY, 14853 and [2] Department of Zoology, University of Oklahoma, Norman, OK.

1) A newly described species, *Hypopomus pinnicaudatus* from French Guiana has a sexually dimorphic caudal filament. Large sexually mature males have long "feathered" tail filaments, females have short cylindrical filaments. The electric organ fills the caudal filaments in each.

2) The electric organ discharge (EOD) differs for the two sexes; male EODs average 1.6 ms in duration, female EODs are 1.1 ms. The power spectra of the male EODs peak at 652 Hz while those from females peak at 915 Hz.

3) The peak-to-peak voltages of the EODs of 34 fish were measured in the natural habitat using a monopolar electrode. The voltage declines according to the inverse square of the distance to the electrical null at the fish's midbody. The voltage increases in proportion to the animal's body length. Females have a higher peak-peak voltage per unit body length than males. Males with damaged tails have a reduced voltage compared to uninjured males of similar size. Males suffer more tail injury than females.

4) A computer model of the EOD suggests that the paradoxical reduction of the amplitude of the male's signal is a consequence of its increase in duration.

5) We hypothesize that sexual dimorphism in *H. pinnicaudatus* has evolved under the influence of sexual selection for long duration pulses. The amplitude reduction resulting from the change in EOD duration is compensated for by the increase in electric organ length, number of electrocytes, and electrocyte size.

Supported by NIMH grant 37972 to CDH, NIMH MB15793 to NC NIH NS19942 to JB.

454.9

SEX DIFFERENCES IN THE CAUDAL FILAMENT, ELECTRIC ORGAN, AND ELECTROCYTES OF *HYPOPOMUS PINNICAUDATUS*. N. C. Comfort, C.D. Hopkins, and A. Bass. Section of Neurobiology and Behavior, Mudd Hall, Cornell University, Ithaca, NY, 14853.

The South American electric fish *Hypopomus pinnicaudatus* (sp. nov.) possesses an electric organ typical among gymnotiform pulse fish in its basic morphology: it extends from the pectoral region to the tip of the tail, and consists of four bilaterally paired columns of drum-shaped electrocytes which diminish in size and spacing as the tail tapers towards the tip. The caudal filament—the portion of the tail caudal to the insertion of the anal fin—is sexually dimorphic in this species. In large (≥ 109 mm) adult males it is long and appears "feathered" in animals captured during the rainy (breeding) season. Females and smaller males have short, cylindrical tails. Caudal filaments of large males contained more electrocytes (55.3 ± .7 rows) than those of females (47.5 ± 1.9 rows) or smaller males (47 ± 4 rows). Single electrocytes in the caudal filament of large males are distinguished in at least four ways. First, they are greater in diameter, averaging 234.9 ± 12.1 µm, compared with 184 ± 6 µm for females, and 184.6 ± 9.3 µm for small males. Second, they are more widely spaced. Third, the electrocyte stalk—which emanates from the posterior face of an electrocyte and receives the cell's innervation—is swollen and triangular in large male fish. Fourth, male electrocytes show greater folding of the anterior face. Despite sex differences in electrocyte number and size, we found no sex difference in the ratio of cell size / cell spacing along the caudal filament, suggesting a sexual allometry in this communication organ. These dimorphisms in electric organ morphology correlate with sex differences in the electric organ discharge, and may represent the result of sexual selection on electric signals. Supported by NIMH Training Grant MH 15793 (NCC), NIH 37972 (CDH), and NIH NS19942 (AB).

454.10

SPIKE DURATION DEPENDS ON STIMULUS DURATION IN ELECTRIC ORGAN OF *STERNOPYGUS*. A. Mills and H.H. Zakon. Dept. Zoology, Univ. Texas, Austin, TX 78712-1064.

The electric organ discharge (EOD) of the gymnotiform wave fish *Sternopygus macrurus* is continually produced as a series of pulses, forming a quasi-sinusoidal waveform. The duration of each pulse is inversely related to EOD frequency; EOD pulse dur. is longer in mature males than in mature females, and this difference can be mimicked by androgen treatment. The basis of the EOD is the simultaneous spiking of the electric organ electrocytes, thus electrocyte spike dur. is expected to be highly correlated with EOD freq. and pulse dur., and to increase in response to androgen.

Recordings from intracellularly-stimulated electrocytes show two types of spikes with different dur., depending on stim. dur; both are similar in appearance to EOD pulses, and have a faster rise to peak than fall to baseline. "Long spikes" (stim.>10 ms) and some "short spikes" (stim. <1.5 ms) were measured from onset to end. Usually the onset of the short spike was obscured by stim. artifact, thus most were measured from peak to end and multiplied by two. Some long spikes showed a pronounced hump in the falling phase not reflected in EOD pulse, which accounts in part for spikes being longer than EOD pulses. Long spikes ranged from 5.2 to 10.0 ms in fish of pulse duration 4.9 to 9.1 ms. The short spikes were an average of 16 ± 11% shorter than the long spikes (N = 13 fish). The relationship to EOD pulse dur. was r = 0.6 (long spikes), and r = 0.3 (short spikes). Spike dur. change with stim. dur. is also seen in vertebrate cardiac fibers, which share with electrocytes the property of continuously firing regularly-spaced spikes. In electrocytes, this may indicate that there is an ion conductance that is activated by a long dur. stim. Since only the long spikes are correlated with EOD pulse dur., it is likely that late ion conductances are important in determining EOD pulse dur

In addition, preliminary studies suggest that long spike dur. in electrocytes increases in response to dihydrotestosterone treatment, resulting in the increase in EOD pulse dur. Similar results are seen in other electric fish species.

454.11

SEXUAL POLYMORPHISMS IN A "VOCALIZING" FISH: SONIC MOTOR AXON TERMINALS. A. Fluet* and A. Bass. (SPON: D. Tapper). Section of Neurobiology & Behavior, Cornell Univ., Ithaca, N.Y. 14853.

The midshipman, *Porichthys notatus*, has two classes of sexually mature males: Type "I" and "II". During the breeding season, both classes of males, along with gravid females, occupy nests. Midshipmen generate sounds by the simultaneous contraction of "drum" muscles attached to the lateral walls of the swimbladder. Only nesting Type I males are known to be sonic and generate a species-typical long duration (up to 1 hr) humming sound. Nevertheless, central brain stimulation shows that Type II males and females can generate a rhythmic sonic discharge with a similar fundamental frequency, although 20% lower than that of Type I males (see Bass & Baker, this mtg.). Type II males also resemble adult females in other traits including muscle fiber diameter and sonic motor axon diameter, both of which are greater in Type I males (Andersen & Bass, this mtg.). This analysis focused on sex differences in nesting animals in the terminal boutons of sonic motor axons. Neuromuscular junctions (NMJs) in all classes share several features including an absence of the postjunctional folds characteristic of mammalian NMJs. As regards differences, the terminal boutons of Type I males: (1) are larger, (2) are often invaginated below the muscle fiber surface, (3) have more Schwann cell wrappings, and (4) lack multiple boutons at single sites along the fiber. Although the differences were not significant at the 0.05 level, synaptic vesicle density decreased in the following order: females, Type II males, Type I males. Synaptic vesicle density was however significantly more in all classes of animals maintained in captivity for 1-4 months beyond the breeding season.

We hypothesize that these differences arise developmentally as a function of both an expanded peripheral target in Type I males and of sex and seasonal differences in the frequency and duration of sonic motor activity. Supported by NSF and NIH.

454.12

COMPARATIVE NEUROCHEMICAL STUDIES OF THE SONIC MOTOR SYSTEM IN TELEOST FISHES. M. Marchaterre, H. Baker, R. Baker and A. Bass. Section of Neurobiol. & Behav., Cornell Univ., Ithaca, N.Y. 14853, Lab. of Molec. Neurobiol., Cornell Univ. Med. Coll., White Plains, N.Y. 10605 and Dept. of Physiol. & Biophys., New York Univ. Med. Ctr.., N.Y., N.Y. 10016.

The Batrachoidiformes includes the oyster toadfish, *Opsanus tau* and the midshipman, *Porichthys notatus*, both of whom generate sound communication signals. The sound's fundamental frequency is determined by the synchronous activity of sonic motoneurons whose firing rate is determined by nearby pacemaker neurons. Our intracellular recording and staining (HRP) studies have suggested the presence of both local inhibitory and excitatory pacemaker neurons. Neurochemical studies were initiated to evaluate known interspecific differences in the sonic motor system. The position of GABA-, serotonin (5HT) and catecholamine- (using tyrosine hydroxylase, TH, antibodies) containing elements were identified in and around the sonic motor nucleus (SMN). Extensive GABA-like immunoreactivity was found in fibers and terminals throughout the SMN in both species. Labelled cell bodies were found in the nucleus, although they were more numerous along the nucleus' lateral margin. Both 5HT and TH fibers were present in the SMN of both species, although less dense than GABA. Both 5HT and TH are more abundant in the SMN of midshipmen although 5HT-containing cells are only present in the SMN of toadfish; however they appear outside the SMN of midshipmen. TH-containing cells surrounded the SMN in both species. The data suggest that GABA plays a major inhibitory role in the sonic discharge of both species. While it is unlikely that either 5HT or catecholamines are excitatory transmitters of the pacemaker system, the data suggest their regional and species variation may contribute to the modulation of the known differences (frequency, amplitude, duration) in sonic motor behavior. Supported by NSF and NIH.

454.13

SEXUAL POLYMORPHISMS IN A "VOCALIZING" FISH: SONIC MOTOR AXON NUMBER AND SIZE. K. Andersen* and A. Bass. (SPON: C. McCormick) Section of Neurobiol. & Behav., Cornell Univ., Ithaca, N.Y. 14853.

The midshipman, *Porichthys notatus*, has a midline sonic motor nucleus (SMN); each half innervates the ipsilateral sonic "drum" muscle which is attached to the lateral walls of the swimbladder. The synchronous firing of sonic motoneurons results in the simultaneous contraction of both muscles and the production of sound. Midshipmen have two classes of sexually mature males: Type "I" and "II". The smaller Type II males are more similar to sexually immature individuals in body size. Nevertheless, Type II males resemble adult females in a number of morphological and behavioral traits including sonic muscle mass and fiber diameter which are several fold greater in Type I males. The sonic discharge frequency in Type I males also exceeds that of females and Type II males which are similar to each other (Bass &Baker, this mtg). This analysis examined possible sex differences in both the number and size (cross sectional area) of sonic motor axons in the occipital nerve which innervates the drum muscles. Average axon number (1700-2500)/side; 0.4-7% difference between sides) was not significantly different between Type I males (n=21) and females (n=26 animals), although it was significantly less (by only 7%) in Type II males (n=25). In contrast, average axon size was 3-fold greater in Type I males (n=13) in comparison to females (n=6) and Type II males (n=6) which were not significantly different from each other. We conclude that: (1) all adult morphs have a similar number of sonic motor axons, (2) the increased sonic muscle mass in Type I males is accompanied by an increase in axonal size rather than number and (3) the differences (Type I vs. Type II & females) and similarities (II & females) in the central sonic discharge rate are paralleled by the profiles of axon size and not number. Supported by NSF and NIH.

454.14

SEX DIFFERENCES IN THE MORPHOLOGY OF PHYSIOLOGICALLY CHARACTERIZED NEURONS IN A "VOCALIZING" FISH. A. Bass and R. Baker. Section of Neurobiol. & Behav., Cornell Univ., Ithaca, N.Y. 14853 and Dept. of Physiol. & Biophys., New York Univ. Med. Ctr., N.Y., N.Y. 10016.

In sound-generating fishes, the sonic motoneuron discharge rate determines the fundamental frequency of acoustic communication signals. Previous studies have shown that midshipmen (*Porichthys notatus*) have two classes of sexually mature males. Large Type "I" males are sonic, but small Type "II" males, like females, are not known to generate sounds. The morphology of central (motoneurons) and peripheral (swimbladder "drum" muscles) elements of the sonic motor system in Type I males differ from those in Type II males and females, which are similar to each other. Sonic muscle mass is, for example, almost six-fold greater in Type I males. Despite these differences, midbrain stimulation demonstrated that females and Type II males could generate a rhythmic sonic discharge similar to Type I males, but distinctly lower (20%) in its fundamental frequency. Intracellular recording and staining (HRP) of 57 pacemaker neurons and motoneurons in 22 animals were carried out to establish the morphological correlates of the above physiological differences. The somata and dendrites of motoneurons and pacemaker cells were 100-300% larger in Type I males. These results demonstrate that a distinct sex difference in sonic behavior is paralleled by a major difference in the morphology of both motoneurons and their presynaptic pacemaker afferents. We further hypothesize that the development of sex differences in the peripheral sonic muscle induces comparable changes in central morphology, but changes in central physiological properties are far less dramatic. Supported by NSF and NIH.

454.15

CARBOCYANINE DYE LABELLED NEURONS AND PROCESSES OF APTERONOTUS LEPTORHYNCHUS PROJECTING TO THE PITUITARY: NEUROCHEMICAL IDENTITY. S.A. Johnston and L. Maler, Dept. of Anatomy, U.of Ottawa, Ontario, Canada, K1H 8M5.

Neurons projecting to the pituitary were retrogradely labelled following application of diI (1,i,dioctadecyl-3,3,3'3'-tetramethylindocarbocyanine perchlorate) crystals to the pituitary of brownghost knife fish perfused with 4% paraformaldehyde. The brain was sealed in a glass container for 4 to 6 wks. at 23°C, in the dark. Vibratome sections (30 um) were photographed through an epifluorescence microscope (rhodamine filter). Cell groups were identified following nissl staining of the sections. Both magnocellular and parvocellular populations were found in the anterior and posterior preoptic nucleus, the anterior periventricular nucleus, the hypothalamus caudal, ventral and lateral, nucleus lateralis tuberis anterior, posterior and nucleus recessus posterior. The nucleus recessus lateral and medial 1, and dorsal hypothalamus had only small cells. The superchiasmatic nucleus had a few large cells. These projections are similar to those in the goldfish (Sloan & Demski, '87) and the mammal (Swanson, '86). Some preoptic neurons contain CRF, vasotocin (magnocellular (n)) and dopamine (Da) and somatostatin (parvocellular (p)). Somatostatin (m & p) and Da(p) have been identified in other hypothalamic hypophysiotropic nuclei (Sas & Maler, '87, '88).

454.16

EVENT RELATED POTENTIALS IN RETINA AND OPTIC TECTUM OF FISH. T.H. Bullock, M.H. Hofmann*, F.K. Nahm, and J.C. Prechtl. Neurobiol. Unit, Scripps Inst. of Oceanog. & Dept. of Neurosci., Sch. of Med., UCSD, La Jolla, CA 92093 and *Dept. Anatomy, Sch. of Med., Univ. of Göttingen, F.R.G.

Compound field potentials were recorded with up to 18 microelectrodes deep in the optic tectum, and with suction electrodes from the distal stump of the cut optic nerve or from the optic nerve head in the opened eye in rays (Rhinobatos, Platyrhinoidis) and teleosts (Leuresthes). Diffuse light flashes of submaximal intensity (I) were delivered in trains with regular or irregular interstimulus intervals (ISI). ERPs are visible in single trials & begin at 50-200 ms after an occasional flash weaker by 20% or more.

One or more stimuli were omitted or the train terminated after various conditioning times (D). Deflections beyond the expected visual evoked potentials (VEPs) to the last flash are called omitted stimulus potentials (OSPs) and considered to be ERPs, without any necessary implication or denial of a temporally specific expectation. OSPs are prominent in single sweeps, even when VEPs have "fatigued" below the ongoing EEG, at ISIs of 300-500 ms or less. They occur approximately on schedule - when the next flash would be due.

Three components of OSP occur alone or in combination: an initial fast peak, a slow wave, and an oscillatory spindle at 25-30 Hz (Rh., 22°C) of 100-700 ms duration. This resembles the OFF response to steady light but OSP latency ca.= ISI + k and changes very little with I or D. The same mean ISI with quasi-random 25-66% jitter gives OSPs with longer latencies and smaller amplitudes, as though the OSP depends on an integrated prediction of ISIs. OSPs also depend on sufficient conditioning, D: under certain conditions (species, brain locus, ISI, I) at least 20 flashes and often more. Some properties of the OSP resemble those of the P300 of humans. It seems unlikely that the OSP requires higher cognitive events, since it is already present in the retina.

454.17

ELECTROSENSORY RESPONSES IN THE GRANULE CELL LAYER OF THE CEREBELLUM OF AN ELASMOBRANCH. J.G. New and T.H. Bullock. Neurobiol. Unit, Scripps Inst. of Oceanog., & Dept. Neurosci., Sch. of Med., Univ. of Calif., San Diego, La Jolla, CA 92093.

Single spikes and multiple unit activity in response to weak (<75 μV) electric field stimuli delivered via a 3 cm dipole perpendicular to the skin surface in the receptive field were recorded with glass micropipettes from the granule cell layer of the cerebellum of immobilized thornbacks (Platyrhinoidis triseriata). The single unit spikes apparently come from large cells, presumably interneurons.

Differences in temporal response properties between two discrete electrosensory regions in the caudal lobe of the cerebellar corpus were studied. Units in a caudo-medial region respond to DC-step stimuli with short latency (60 ms) rapidly adapting bursts following stimulus onset & offset. The intensity, latency and duration of these responses are similar regardless of stimulus polarity, although an asymmetry between onset & offset bursts is frequently observed. Such units respond to low frequency (<10 Hz) sinusoidal stimuli with bursts of spikes on each rising as well as falling phase.

Units in a rostromedial region exhibit polarity-specific excitation or inhibition with excitation following cathodal stimuli and inhibition after presentation of an anode. Excitatory responses consist of more slowly adapting bursts of activity after stimulus onset, with inhibition of similar latency and duration following offset. Units in this area respond to low frequency sinusoidal stimuli with excitation during the negative phase peaking at maximum amplitude and inhibition during the positive phase.

Units in both regions were unimodal, exhibiting no response to visual or tactile stimuli. The results of this study indicate that two discrete electrosensory regions in the caudal lobe of the cerebellar corpus respond to distinctly different aspects of electric field stimuli. The electrosensory afferent innervation of these areas is unknown.

Supported in part by NIH NS08114 to JGN.

454.18

ELECTRORECEPTIVE AND PROPRIORECEPTIVE REPRESENTATIONS IN THE DORSAL GRANULAR RIDGE OF SKATES. R. A. Conley* and D. Bodznick Wesleyan Univ. Biology Dept., Middletown, CT 06457

Physiological responses and receptive fields of proprioreceptive and electroreceptive neurons in the dorsal granular ridge (DGR) of *Raja erinacea* have been studied in decerebrate, curarized fish. DGR is part of the vestibulolateral cerebellum in elasmobranchs and projects topographically via parallel fibers to the electrosensory dorsal octavolateralis nucleus (DON) in the medulla. DGR probably has a major influence on primary electrosensory processing, yet its role is unknown. Of 178 neurons recorded in a blind mapping study, 99 (56%) had their discharges modulated when the pectoral fin was displaced and 79 (44%) were unaffected by fin displacement, but responded to DC electric fields. No bimodal units were encountered. Units were characterized as proprioreceptive since they increased their firing rate tonically in proportion to fin displacement, although some units had a phasic component during fin movement. For each proprioreceptive unit there was a limited area of sensitivity along the fin margin. The electroreceptive units had large ipsilateral receptive fields, whose exact boundaries were often unclear. But at threshold, nearly all units had a best area within the receptive field. In general, both proprioreceptive and electroreceptive units showed a progression of receptive fields from posterior to anterior body along the caudorostral axis of DGR. DGR forms a topographic projection onto DON such that caudal DGR projects to dorsal DON and rostral DGR projects to ventral DON. As a caudal to rostral body map is present along the dorsoventral axis of the DON, these results suggest that DGR's projection onto the DON is homotopic.

454.19

AUTORADIOGRAPHIC LOCALIZATION OF DIHYDROTESTOSTERONE CONCENTRATING NEURONS IN THE BRAIN OF THE OYSTER TOADFISH. M.L. Fine and D.A. Keefer*. Depts. of Biology, Virginia Commonwealth University, Richmond, VA 23284 and Loyola College, Baltimore, MD 21210.

Steroid-concentrating neurons in the brainstem have been reported in two teleosts, the toadfish and a mormyrid, both of which produce courtship sounds. In this study ^{3}H DHT-labelled neurons were found in the forebrain and brainstem. Positive sites included the supracommissural nucleus of the ventral telencephalon and the base of the lateral portion of the dorsal telencephalon. Small numbers of cells were labelled sporadically in the parvo- and magnocellular preoptic nucleus and in the ventral and dorsal hypothalamus. The caudal hypothalamus had a densely-labelled triangular-shaped nucleus and nearby scattered cells. The periventricular portion of the posterior tuberculum of the thalamus was also labelled. Scattered heavily labelled cells were present throughout the basal optic tectum and superficial torus semicircularis. In the medulla a dorsal accumulation of cells was present beneath the cerebellum on either side of the fourth ventricle rostrally and in reticular neurons caudally.

Compared with an earlier study with ^{3}H estradiol, estrogen labelling is generally more prominent in the forebrain, and with the exception of the torus semicircularis and tectum, no estrogen target neurons have been found in the brainstem. Supported by NIH MH38921 and NSF PCM8309144.

454.20

DESCENDING CONTROL OF ELECTROSENSORY PROCESSING

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The electrosensory lateral line lobe (ELL), the first-order electrosensory processing station, receives major descending inputs in addition to afferents from the electroreceptors. The nucleus praeeminentialis (NP), which receives electrosensory inputs from the ELL and the torus semicircularis, is the principle source of descending input to the ELL. NP neurons were studied using extracellular and intracellular recording methods and Lucifer Yellow labeling for anatomical identification focusing on two categories of neurons.

Stellate (ST) neurons show no spontaneous activity, respond to stepwise changes in electric organ discharge (EOD) amplitude phasically and are insensitive to sinusoidal modulations of EOD amplitude of frequencies exceeding 30 Hz. Previously reported as the most common NP cell type, ST neurons respond vigorously to electrolocation targets moving along the contralateral side. Ipsilateral targets cause little or no response, hence the ST neurons receive excitatory input from the contralateral ELL. We found two populations of ST neurons, one responds to contralateral increases in EOD amplitude while the other responds to decreases, there were no differences in morphology or projection pattern.

Tufted neurons influence the ELL indirectly via their projection to the posterior eminentia granularis (EGP). These fire spontaneously at 60 - 80 spikes/s, have bilateral receptive fields, respond to EOD AMs in excess of 64 Hz, and respond tonically to prolonged changes in EOD amplitude. Examples of these have been seen which alter their firing rate by as much as 60 spikes/s per 100 μV change in transepidermal voltage. Earlier studies have shown that the sensitivity of ELL output neurons changes to compensate for alterations in EOD amplitude and the associated changes in receptor afferent input. The tufted cells may provide information about changes in EOD amplitude needed for this gain control mechanism to function. Supported by NIH Grant NS12337 and OCAST #1669

454.21

MAGNETIC ORIENTATION IN NORMAL AND TELENCEPHALON-ABLATED RAINBOW TROUT (Salmo gairdneri). G.L. Chew* (SPON: P. Ahluwalia). Dept. of Psychol., University of Lethbridge, Lethbridge, AB, Canada, T1K 3M4.

The orientation of juvenile rainbow trout (Salmo gairdneri) (age approx. 6 months) was tested under: (1) normal, (2) null, and (3) rotated magnetic field conditions. In the normal geomagnetic condition (magnetic field strength ≈ 0.5 Gauss), juveniles showed a statistically significant mean orientation in alignment with geomagnetic north. In contrast, individuals tested in a null magnetic environment were only randomly oriented. Rotation of the horizontal component of the geomagnetic field using a Rubens coil array also shifted the mean orientation in the direction of rotation although this relationship is nonlinear.

A second group of trout, with their dorsal telencephali removed, were also tested. Mean group orientation in the normal and rotated magnetic field conditions were, as in the null field case, random. These findings show: (1) freshwater salmonids are capable of using magnetic orientation in spatial behaviour, (2) the horizontal component of the local magnetic field vector is involved in such orientation, and (3) normal magnetic orientation is not possible without the dorsal telencephalon which contains the lateral zone of the dorsal telencephalon, a piscine hippocampal homolog.

454.22

ACOUSTIC PRESSURE ACTIVATION OF STARTLE IN THE RESTRAINED GOLDFISH. J.G.CANFIELD AND R.C.Eaton. Neuroscience Group, Univ. of Colorado, Boulder, CO 80309-0334.

In the Mauthner cell, we have identified an auditory EPSP which has a latency of 3.5 - 4.5 ms and exhibits a complex waveform to air-mediated sound. This auditory potential can be recorded extracellularly (EC) near the cell soma and intracellularly (IC) in both the soma and axon at the level of the vagal lobes. The axon-recorded potential disappears if the cell soma is damaged. The amplitude of either the EC- or IC-recorded EPSP declines with increasing background noise and the EC field remains intact when the posterior lateral lines are severed but declines when the swim bladder is deflated. A similar auditory EPSP has been recorded from other reticulospinal axons which fire at short latency to spinal stimulation.

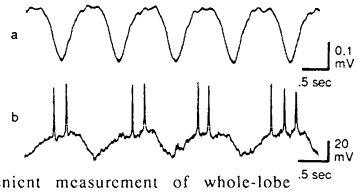
We have also been able to initiate startle responses in an anesthetized, restrained, but unparalyzed preparation by application of air pressure pulses to the body surface dorsal-lateral to the anterior chamber of the swim bladder. This stimulus also elicits a similar EC field response.

Since acoustic stimulation mediated by the swim bladder appears to cause a large field potential and initiate a startle response, it may be that ostariophysine fishes normally use this input to trigger escape whereas the near-field displacement component of the acoustic stimulus is superimposed to code response trajectory. We also postulate that the response trajectory results from bias on the output of the system by similar auditory input to other reticulospinal neurons. [Supported by NIH grant NS22621].

455.1

ODOR-MODULATED NETWORK OSCILLATIONS IN THE PROCEREBRAL LOBE OF *Limax*. A. Gelperin, D. W. Tank, J. Flores*, L. Rhines*. AT&T Bell Laboratories, Murray Hill, N. J. 07974

We find that odor stimulation of the nose modulates the spontaneously oscillating field potential of the procerebral (PC) lobe. Using a nose-brain preparation, brief pulses of moist air or 2-ethyl-3-methoxypyrazine were delivered to the nose while the PC oscillation was recorded with a saline filled electrode in the PC neuropil. Odor pulses modulate the spontaneous oscillation, resulting in transient DC shifts and changes in frequency and waveform of the oscillation. Air pulses also modulate the oscillation, but to a lesser extent than odor pulses. Simultaneous field potential recordings from distal cell body and basal neuropil regions show in phase oscillations of opposite polarity. A suction electrode placed over the intact lobe provides a convenient measurement of whole-lobe oscillation(Fig. 1a). Intracellular recordings show that individual neurons generate action potentials during the depolarizing phase of large membrane potential oscillations (Fig. 1b). It will be of great interest to determine if there exist odor-generated spatial patterns of excitation in PC neurons.



455.2

CHOLINERGIC ANTAGONISTS BLOCK THE LYMNAEA FEEDING RHYTHM. C.J.H. Elliott * (SPON : G.P. Ferguson. Dept. Biol., Univ. York, York, YO1 5DD, U.K..

In the isolated CNS of the pond snail, Lymnaea stagnalis, the feeding rhythm persists at a slow rate, through the interaction of the N1, N2 and N3 premotor interneurones (Elliott, C.J.H. & Benjamin, P.R., J. Neurophysiol. 54:1396, 1985).

Phenyltrimethylammonium (PTMA, 0.5mM in normal saline) blocks all synaptic output from the N1 neurones and rhythmic activity then appears to be confined to these interneurones.

Hexamethonium or atropine (0.5mM) block the EPSPS (but not the IPSPS) produced by the N1 interneurones. These antagonists also abolish the spontaneous rhythm and slow the rhythm elicited by the stimulation of a modulatory interneurone, the SO.

These results confirm that the N1 neurones are cholinergic (Elliott, C.J.H et al., Symp. Biol. Hung. 36:697, 1988) and suggest that recurrent inhibition between the N1 and N2 neurones plays an important part in rhythm generation.

455.3

SWALLOWING IN THE NUDIBRANCH *MELIBE LEONINA* IS CONTROLLED BY THE BUCCAL GANGLIA. W.H. Watson III, A. Vetrovs, and J.R. Trimarchi, Zoology Department, Univ. of New Hampshire, Durham, N.H. 03824.

Molluscan buccal ganglia have been used extensively to study the neural basis of feeding behavior. However, detailed information about the underlying neural circuits is lacking for most buccal preparations because of their size and complexity. We have been investigating the buccal ganglia in the nudibranch, *Melibe leonina*, because their simplicity (30 neurons) makes them amenable to analysis at the cellular level. The goals of our initial studies were to: 1) describe the feeding behavior in detail; 2) quantify the influence of food availability on the feeding rate and; 3) determine the role of the paired buccal ganglia in feeding.

Melibe has a unique feeding behavior because it lacks a buccal mass. It captures food by opening and closing its oral hood at a frequency of 1 - 4 episodes per minute. The more prey, the faster the capture rate. When prey enter the outstretched oral hood it closes and the edges of the hood are brought together so that tentacles lining the inner rim of the hood form a sieve. Then the oral hood contracts, water is forced out through the tentacles and zooplankton are filtered from the water. Finally, the tentacles transport the food into the mouth and it is swallowed by peristalsis of the esophagus.

To examine the role of the buccal ganglia we removed them from a group of 10 *Melibe*, and compared their feeding with groups of sham-operated and control animals. The three groups were allowed to feed for 5 days in aquaria containing prey which could be easily recognized during subsequent analysis. Then animals were dissected and their gut contents analyzed. All animals captured food normally and ate comparable numbers of prey, indicating that the buccal ganglia do not play a role in food capture. Analysis of the distribution of food in the gut revealed that in lesioned animals up to 80% of the food ingested remained in the anterior esophagus. In contrast, in both control groups the majority of the food was in the stomach. These data indicate that the buccal ganglia in *Melibe* control swallowing, but not the capture of food.

(Supported by grants from CURF to A. V, UROP to J.T., and Hubbard W.W.)

455.4

AN IDENTIFIED CEREBRAL NEURON PROMOTES MULTIPLE EFFECTS ASSOCIATED WITH FOOD-INDUCED AROUSAL IN *APLYSIA*. T. Teyke*, K.R. Weiss and I Kupfermann. Center Neurobiology & Behavior, Columbia University and NYS Psychiat. Inst., New York, NY 10032.

The food-induced arousal state in *Aplysia* has multiple manifestations, including changes in consummatory behaviors (increased rate and magnitude of biting), appetitive behaviors (head-up posture), autonomic responses (increased heart rate), and defensive responses (depressed head withdrawal). To determine if these responses might be controlled by a central arousal system, we examined the neural control of appetitive arousal, which appears to precede all the other aspects of arousal. The role of neurons in the cerebral ganglion which project to the pedal ganglion was studied. A unique cerebral-pedal interneuron, CPA, was identified, which activates numerous neurons in the pedal ganglion. The CPA has either poly- or monosynaptic connections to more than 50% of the pedal ganglion neurons, including a large population of neck motor neurons. Firing the CPA evokes bilateral contractions of neck muscles, which could serve to lift the head of the animal into the feeding posture. The activity that the CPA evokes in the pedal ganglion provides excitatory drive to a number of other neurons which mediate various aspects of the food arousal state, including the MCCs, which modulate biting; the CBIs, which drive the biting program; and the RB cells, which increase heart rate. Finally, pedal ganglion activity driven by the CPA inhibits cerebral Bn cells, which mediate head withdrawal. Stimulation of the tentacles with seaweed excites the CPAs, with concomitant excitation of the MCCs, and inhibition of the Bn cells. Removing the CPA neurons from the circuit by hyperpolarizing them bilaterally, strongly reduced the effects of seaweed on both the MCC and the Bn cells. Therefore, it appears as if activity of the CPA may be necessary for some, and perhaps most aspects of the responses associated with the food-induced arousal state. It is possible that the CPA functions in the role of a command neuron or command element that evokes a behavioral state in the animal, rather than a specific behavior.

455.5

ESCAPE BEHAVIOR IN JUVENILE APLYSIA IS AFFECTED BY PROTEIN AND TRYPTOPHAN DEFICIENT DIET. S.Kurtz*, J.Flinn, R.Holt* J.Weber*, & K.Allen*, Dept. of Psych, George Mason, Fairfax, VA 22030.

In two studies Aplysia c. were fed from 50 to 110+ days post-hatching on either a protein & tryptophan rich Gracilaria or on a low protein & tryptophan Enteromorpha diet. At 110+ days Aplysia were tested for short-term sensitization, using escape locomotion following tail shock. The experimental paradigm consisted of two weak shocks 1.5x the current required for tail withdrawal, followed by the sensitizing shock and two more weak shocks. Number of steps in the two min. following each weak shock measured activity. No differences were seen between feeding conditions for tail withdrawal threshold but significant differences were found both in the baseline motor activity & in the level of short-term sensitization. Aplysia raised on Enteromorpha showed significantly higher levels of baseline activity than siblings raised on Gracilaria, $t(35)=2.24$ p .05 for study 1, & $t(42)=1.89$, p .05 for study 2. This result was surprising since tryptophan is the precurser to serotonin which triggers escape behavior (Mackay & Carew, J.Neurosci 3:1469,1983). As hypothesized Aplysia raised on Enteromorpha showed less sensitization than those raised on Gracilaria, as shown by ANOVA & planned comparisons ($t(35=1.78$ p .05 for study 1, & $t(42)=1.90$ p .05 study 2.) These results show that feeding regimes during development can affect both escape behavior and level of learning.

455.6

SEROTONIN AFFECTS BEHAVIORAL STATE AND FEEDING BEHAVIOR IN THE SNAIL, HELIX ASPERSA, BUT HAS NO EFFECT ON SEXUAL BEHAVIOR. S.A. Adamo and R. Chase. Department of Biology, McGill University, Montreal, Canada H3A 1B1.

The degree of activity in H. aspersa can be used as a measure of the snail's behavioral state. Behavioral state can be taken to represent an underlying level of general arousal, as has been done for Aplysia and Pleurobranchaea. Injections of serotonin (10 -7 moles/kg body weight) increased behavioral state scores in H. aspersa ($p < 0.01$), which is consistent with the effects of serotonin in Aplysia and Pleurobranchaea (Palovcik et. al., Behav. Neural Biol., 35:383, 1982). Handling the animals had a similar, but smaller effect ($p < 0.05$). Serotonin injections also increased feeding behavior as compared to controls ($p < 0.05$).

Serotonin had no effect on sexual arousal (monitored by observing changes in the snail's genital eversion). Serotonin also had no effect on sexual proclivity (i.e. the frequency of mating, $p >> 0.05$).

These data suggest that, in H. aspersa, mechanisms that mediate sexual behavior are distinct from those that control feeding and behavioral state. Therefore, sexual behavior is one 'motivated' behavior that does not appear to be significantly modulated by a postulated gastropod central serotonergic system (Palovcik et. al., 1982).

455.7

Mechanosensory Inputs Produce a New Type of Nonassociative Learning in Tritonia. G.Brown* and P.A.Getting (SPON: C.Cleland). Neuroscience Program, University of Iowa, Iowa City, IA 52242.

Previously it was thought that in Tritonia, reponses to mechanical and chemical stimulation were mediated by the same population of afferents. The "S-cells" which trigger escape swimming due to chemical input also show a brief phasic response to mechanical stimulation. Three recent observations however suggest that responses to mechanical and chemical stimuli are mediated by distinct populations of neurons: 1) Chemical stimulation at a site distal to the gills elicits gill withdrawl whereas mechanical stimulation at the same site elicits gill extension. 2) When a chemical stimulus (.15 ml of 4M NaCl applied to the tail) which normally (>95%) elicits swimming was given together with a mechanical stimulus (25 pokes to midbody region with a glass probe), swimming was not observed in 9 of 10 animals. 3) When the mechanical stimulus was given after the initiation of swimming, the duration of the swim was attenuated (N=11, p<.001).

Mechanical stimulation also produces longer lasting inhibition of the response. Animals were trained with ten trials of mechanical stimulation at ten minute intervals and tested with the chemical stimulus 30 min after the 10th trial. Six of 18 animals did not swim when given the chemical test stimulus, compared to 1 of 23 in animals which received no mechanical stimulation (p<.05).

More dramatic results were seen when training consisted of both mechanical and chemical stimulation. Control animals received 10 chemical stimuli at 10 min intervals and were then tested with the same stimulus 30 min later. All these animals swam to the test stimulus (11 of 11). However, when 10 mechanical stimuli were interspersed with the chemical stimuli, 8 of 14 animals failed to swim (p<.02). In addition, the 6 animals which did swim had a longer onset latency than controls (10.4 vs 5.0 s, p<.01). This long lasting inhibition of the swim response due to mechanical stimulation represents a new form of nonassociative learning in Tritonia. Supported by NS17325 and NS07247.

455.8

FMRFamide ACTS DIRECTLY ON ISOLATED APLYSIA GILL MUSCLE CELLS. D. Cawthorpe* & K. Lukowiak (SPON: K.E. Cooper). University of Calgary, Calgary, Alberta T2N 4N1 Canada.

Aplysia californica is a model system used in the study of the neural and biochemical basis of behavior. Both the central nervous system (CNS) and peripheral nervous system (PNS) have been shown to act in an integrated manner to mediate gill withdrawal behavior (GWR). This GWR has been shown to undergo both associative and non-associative forms of learning. To date most studies have concentrated upon the role of the CNS in learning due to the easier accessibility of the CNS pathways. As yet little is known about the PNS. The particular sites of action and the identity of neurotransmitters and/or neuromodulators in the PNS remain largely unknown. In response to this gap in our knowledge about the PNS, a primary culture of dissociated gill muscle fibers has been developed. This provides an opportunity to survey the neurotransmitters and neuromodulators known to act in the PNS. We report here that the endogenous peptide, FMRFamide, acts directly on isolated gill muscle fibers to bring about contractions at concentrations (10 nM) which have been shown to increase the GWR amplitude and prevent habituation in the in vitro reduced gill preparation (Cawthorpe et al, 1988; Higgins et al, 1989).

455.9

NEUROSECRETORY CELL R15 IN *APLYSIA* ACTIVATES RESPIRATORY PUMPING, MOTONEURON L7, AND THE HERMAPHRODITIC DUCT A. Alevizos, K. R. Weiss, and I. Koester. **Center for Neurobiology & Behavior, Dept. of Psychiatry, and Dept. of Physiology and Cellular Biophysics, Columbia University, N.Y., NY**

R15 is a neurosecretory cell in the abdominal ganglion, thought to be involved in water and electrolyte regulation. It also has central synaptic effects in the abdominal ganglion - exciting unidentified cells of the left lower quadrant and the LBvc vasoconstrictor cells. We describe here the modulatory effects of R15 and its bioactive α-1 peptide on 1) respiratory pumping 2) multimodal motoneuron L7 and 3) peristaltic activity of the large hermaphroditic duct.

Respiratory pumping consists of transient, synchronous pumping actions of the mantle organs and parapodia. It is driven by a network of about 30 electrically and chemically coupled interneurons in the abdominal ganglion. We found that if R15 is hyperpolarized for prolonged periods of time (1.5 - 2 hours) to prevent it from firing, and then allowed to burst spontaneously for 5-60 minute intervals, the basal frequency of respiratory pumping exhibits an increase of 2- to 6-fold, which gradually decays over 20 to 60 minutes. In the same long hyperpolarization protocol, R15 also excited the multimodal cardio-respiratory motoneuron L7. Dye-filling the R15 soma revealed that it also innervates the large hermaphroditic duct. With the hyperpolarization protocol described above, we found that R15 bursting generates peristalsis of the portion of the hermaphroditic duct involved in egg laying. All 3 of these effects of R15 are mimicked by the R15α-1 peptide. The R15α-1 peptide effects exhibit pronounced desensitization that lasts for hours, and all the effects of R15 can be cross-desensitized by previous application of the peptide. All the effects of R15 described above are robust, present in all preparations, and persist in solutions that block polysynaptic pathways, suggesting that R15 mediates these effects by direct action on its targets.

Our results suggest that R15, by virtue of its central and peripheral connections, may integrate several behavioral components of oviposition.

455.10

ACTIONS AND DISTRIBUTION OF THE ALTERNATIVELY SPLICED R15 PEPTIDE OF *APLYSIA CALIFORNICA*. D. Karagogeos, A. Alevizos, K. R. Weiss, L. B. Buck*, and I. Koester **Center for Neurobiology and Behavior, Dept. of Psychiatry, H.H.M.I and Dept. of Physiology, Columbia University, N.Y., N.Y.**

Buck et al. (1987) have characterized the cDNA of the major neuropeptide expressed in R15. This neuron, a well studied bursting cell in the abdominal ganglion, is involved in a variety of behaviors (Alevizos et al., these abstracts). The polyprotein precursor of this neuropeptide can be cleaved at five sites to produce a variety of peptides. The mRNA encoding the polyprotein can be spliced alternatively in different neurons, to produce an even larger diversity of peptides.

Using a three stage HPLC analysis, we showed that the alternatively spliced peptide (R15α-2; 24 amino acids long) is synthesized in the abdominal ganglion of *Aplysia* by as yet unidentified cells. Furthermore, the peptide was found to be bioactive on a variety of organs. It causes cardioexcitation, shortening and/or constriction of all the major arteries, contraction of the main hermaphroditic duct, the gill and esophagus.

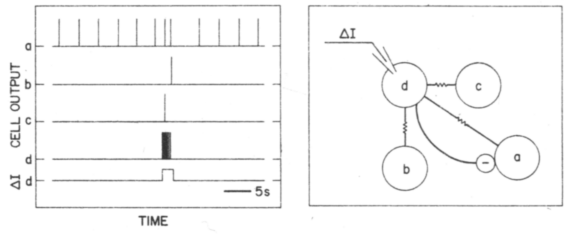
We have raised a rabbit antibody against the R15α-2 peptide to study its distribution in the CNS and the peripheral organs of the animal. Since the alternative splicing affects only a small portion of the peptide, the antibody recognizes both the R15α-1 and R15α-2 peptides. Immunocytochemistry showed that the antibody is highly selective, staining about 20 cells in the total CNS. There is extensive innervation of a variety of peripheral organs by immunoreactive terminals, including the main arteries, the large hermaphroditic duct, the sheath of the hepatopancreas, the pericardium, and the base of the auricle of the heart. Dye fillings of R15 showed that some of these organs are sites of R15 innervation.

Current work is aimed at identifying individual cells that synthesize the peptide and describing their physiological outputs.

455.11

Synaptic interactions in assemblies of cultured *Aplysia* neurons. I: Analysis of optically recorded electrical activity. D. Kleinfeld, T. D. Parsons, F. Raccuia-Behling*, A. L. Obaid, and B. M. Salzberg. University of Pennsylvania School of Medicine and AT&T Bell Laboratories.

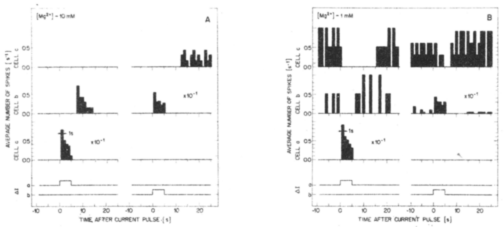
We constructed ensembles of *Aplysia* LUQ neurons in culture. These cells form chemical connections and may couple electrotonically. Their electrical activity was measured with multisite optical recording techniques and voltage sensitive dyes (Parsons *et al.*, Biophys. J., July 1989). These techniques allowed us to record for prolonged periods with a good signal-to-noise ratio and negligible phototoxicity (2 hr, S/N ≃ 10). However, the extensive outgrowth of the neurons caused each detector to monitor the activity of many cells. We show how individual spike trains were reconstructed from the spatial distribution of timing and amplitude information in the optical records. The effective connectivity was determined from the spike trains (Figure). Weak interactions could be deduced from repeated measurements. (Supported by AT&T and NS 16824)



455.12

Synaptic interactions in assemblies of cultured *Aplysia* neurons. II: Dynamic patterns of effective connectivity. T. D. Parsons, B. M. Salzberg, A. L. Obaid, F. Raccuia-Behling*, and D. Kleinfeld. AT&T Bell Laboratories and University of Pennsylvania School of Medicine.

We studied the *effective* connectivity between LUQ neurons co-cultured as 4 to 6 cell ensembles. These cells form biphasic chemical connections, with inhibition followed by excitation, as well as monophasic inhibitory connections. The effective connectivity was determined (preceeding abstract) when the neurons were quiescent ($[Mg^{2+}] \simeq 10$ mM) and when they were firing at ~ 0.5 Hz ($[Mg^{2+}] \simeq 1$ mM). Under conditions of quiescence the neurons exhibited excitatory interactions (Histogram A). In contrast, the same ensemble exhibited slow inhibitory connections when the cells were active (Histogram B). The change in effective connectivity could be understood in terms of the underlying monosynaptic connections. The excitatory phase dominated when the neurons were quiescent while inhibition dominated when the neurons were active. Similar mechanisms for dynamic changes in effective connectivity may occur *in vivo*. (Supported by AT&T and NS 16824)



455.13

EVIDENCE FOR ASSOCIATIVE LEARNING IN THE NEMATODE *C.ELEGANS* N. Kumar*, M. Williams*, J. Culotti† and D. van der Kooy, (SPON: A. Roach), Dept. of Anatomy and †Medical Genetics, Univ. of Toronto, Toronto, Canada, M5S 1A8.

A simple *in vivo* model for associative learning and memory offers many advantages in the search for the molecular basis of of these processes. The nematode, *C. elegans*, seems to be an ideal model system for associative learning because of its well characterized genetics, neuroanatomy and development. We developed a classical conditioning paradigm using chemotaxic responses to the conditioned stimuli (CS) Na^+ ($NaCH_3OO$) and Cl^- (NH_4Cl) ions, and *E. coli* (a food source) as an unconditioned stimuli (UCS). CS levels were balanced such that naive animals displayed equal preferences for either CS. The animals were then trained by exposing them to one ion paired with the UCS (CS+) followed by exposure to the other ion in the absence of the UCS (CS-). The experiment was counterbalanced for which CS was paired with the UCS and for order of presentation. The testing consisted of placing approximately 100 of the trained animals between point gradients of each CS (in the absence of the UCS) and allowing them to migrate towards one of the gradient centers. Immediately after one CS+ and one CS- pairing, 67.1±3.1% of the animals demonstrated a preference for the CS paired with the UCS. A five hour food deprivation prior to training increased the level of learning to 79.6±4.7%. These animals are also capable of retaining the association, as learning levels were still at 62.3±5.5% 7 hours post-training. By 27 hours post-training the level of learning was no longer significantly different (56.5±9.8 %) from controls. We have also been able to demonstrate conditioned aversion learning by replacing *E. coli* as the UCS with an aversive garlic extract. In this case nematodes can be trained to avoid the UCS paired CS (63.8±2.1 %). Thus, the nematode is capable of associative learning and should be useful in a mutational analysis of the molecular basis of learning and memory.

455.14

MALE-MATING DEFECTIVE MUTANTS OF *C. ELEGANS* K. Liu* and P. Sternberg* (SPON: S. Benzer). Div. of Biology, 156-29, Caltech, Pasadena, CA 91125.

To understand how genes specify an innate behavior, we are genetically dissecting male-mating in *C. elegans*. Since the hermaphrodites are self-fertilizing, mutations that affect or even eliminate male-mating behavior can be isolated and maintained for study. By mutagenizing with EMS a strain that segregates 40% XO males via non-disjunction of the X-chromosome, we have isolated mutants that have no immediately visible anatomical defects yet are copulation defective (Cod). We predict that such mutants might be blocked in mating behavior at any one of a number of neccesary steps: attraction to hermaphrodites, response to contact with hermaphrodites, location of the vulva, insertion of spicules into vulva, and sperm transfer.

Of the mutants isolated thus far, the majority (6 of 9) appear to be unable to insert their spicules, indicating a muscle or motor defect. One responds poorly to contact with hermaphrodites and two are unable to locate the vulva. These failures may indicate underlying sensory defects. In addition, two mutant strains give males that sire very few progeny, indicating either a defect in sperm transfer or sperm potency.

455.15

LEVER-PRESS CONDITIONING IN THE CRAB. Charles I. Abramson* and Richard D. Feinman, Dept. of Biochemistry. SUNY Health Science Center. Brooklyn, NY 11203.

Operant conditioning is the least well investigated learning procedure in animals with simple nervous systems. In particular, lever press and other traditional operant behaviors studied in vertebrates have been difficult to obtain in invertebrate species. We have developed an operant chamber for the green crab *Carcinus maenas* in which pressing a lever produces food reward. The operant chamber, submerged in seawater, contained a plastic rod attached to a microswitch and food was dispensed from a tube positioned at mouth level. In one series of experiments, two bars were presented on either side of the food tube. Pressing one lever (S+) was reinforced whereas, the other (S-) was not. Experimental animals pressed S+ with an average rate of 6 min^{-1} over the first 5 min and then gradually slowed down. Responses to S- averaged 3 min^{-1} and rapidly dropped out. Typical total responses for 20 min sessions were 75 responses to S+ and 25 to S-. (Naive animals placed in the chamber with the food dispenser turned off pressed the bars no more than 10 times in a 20 minute session without obvious discrimination). When the contingencies associated with the lever were reversed animals persisted in pressing the old bar (now S-) on the first day but learned to switch to the correct bar on the second. Trained animals maintained high rates of responding on a fixed ratio of 2 schedule (FR2, every other response reinforced) or even an FR3. In a second series of experiments, one group of animals was presented with a single bar and an FR1 schedule. A second group in a similar chamber was "yoked" to the first — received reinforcement whenever the first group did: effectively a variable time schedule. This non-contingent schedule caused a decrease in performance to one third the rate of the FR1 group. The performance of crabs in the "Skinner box" shows discrimination and dependence on the contingencies of reinforcement. (Supported by grant BNS 8819830 from the National Science Foundation and funds from the Research Foundation of the State University of New York).

455.16

POSTSYNAPTIC INHIBITION OF THE LATERAL GIANT NEURON DURING RESTRAINT-INDUCED SUPPRESSION OF CRAYFISH ESCAPE. E.T. Vu and F.B. Krasne. Neuroscience Program, Dept. of Psychology, and Brain Research Institute, University of California, Los Angeles, CA 90024.

The suppression of the crayfish lateral giant (LG) escape response by restraint is at least partially mediated by decreased synaptic transmission onto the LG. We have previously reported a 15% decrease in root-evoked LG postsynaptic potentials (PSP). We now report comparable decreases in both the electrical unitary PSP from the identified sensory interneuron, interneuron A, and in polarizing pulses injected into the electrically coupled contralateral LG. In contrast, axonally applied LG polarization is only decreased about 1%, thus suggesting that the LG receives a conductance increasing postsynaptic inhibition during restraint that operates distally in the dendrite. This site of action is different from that of recurrent inhibition of this cell, and is similar to that of another recently reported form of postsynaptic inhibition of the LG, post excitatory inhibition. Supported by USPHS grant NS08108 (FK) and a Predoct. NSF Fellowship (EV).

455.17

EFFECTS OF OCTOPAMINE ON UNITARY EPSPS IN A FIRST ORDER INTERNEURON OF CRAYFISH LATERAL GIANT ESCAPE CIRCUIT. J. Bustamante* and F. Krasne. Dept. Physiology, Faculty of Medicine, Universidad Complutense de Madrid and Dept. Psychology and Brain Research Institute, UCLA.

The excitability of the crayfish lateral giant escape reflex can be increased for hours by traumatic stimulation, LTP-like regimens, and exposure to octopamine. Viewing the octopamine augmentation as a possible model of the other types, we examined octopamine's effect on unitary EPSPs in interneuron A, the largest first order interneuron of the circuit. Octopamine exposure caused EPSP amplitude to increase (50%, SD 28%) within minutes, but recovery required hours of washout. Augmented EPSPs had more variable amplitudes (control cv of 7.8% increased by 56%, SEM 18%), peaked later, and decayed more slowly (4.4 ms half fall increased significantly by 5%) than controls. The results suggest a model in which octopamine brings into increased action a population of previously unexpressed synaptic transmission sites that have relatively variable release and slow decay kinetics (t approx 20 compared to 6 for control). Supported by CSIC Fellowship, Spain (JB) & NIH grant NS 08108 (FK).

455.18

ALTERED SYNAPTIC TRANSMISSION AND MEMBRANE CURRENTS IN THE *DROSOPHILA* LEARNING MUTANT *dunce* Yi Zhong* & Chun-Fang Wu (Spon: S. Singh). Dept. of Biology, University of Iowa, Iowa City, IA 52242

Several proposed models for cellular mechanisms underlying learning and memory involve regulation of neuronal plasticity by second messenger systems. A general question can be raised as to whether a discrete set of genes controls the molecular regulatory mechanisms exclusively required for learning and memory. Mutations of the *dunce* (*dnc*) locus in *Drosophila*, which cause reduced phosphodiesterase II activity resulting in elevated cAMP levels, reveal deficiencies in learning and memory paradigms but not in other behavioral tests. It is important to know whether *dnc* mutants affect synaptic plasticity and membrane currents and whether the effects are confined to subsets of neurons or more generally to excitable cells.

With the two-microelectrode voltage-clamp method, we examined neuromuscular transmission and muscle membrane currents in larval body wall muscle preparations. The excitatory junctional currents (ejcs) in *dnc* showed increased quantal contents with miniature ejcs of normal amplitude. With 0.2 mM Ca^{++} in saline, **facilitation** was induced by 4 Hz stimulation in normal larvae, but not in *dnc*. At 0.4 mM Ca^{++}, 4 Hz stimulation caused a significant **depression** in *dnc* in contrast to facilitation in normal. In addition, following 5 Hz stimulation for 2 min, **potentiation**, which lasts for minutes, was observed in normal, but not in *dnc*. Furthermore, we found an increase in the amplitude of both transient K^+ current (I_A) and inward Ca^{++} current in *dnc* muscle membrane. Interestingly, modification of synaptic efficacy and of these two currents has been implicated in other invertebrate model systems for learning and memory.

Supported by NIH grants NS 18500 and NS 26528

455.19

SEROTONIN DEPLETION REDUCES SEVERAL FORMS OF BEHAVIORAL FACILITATION IN THE LEECH. T. Karrer, J. Erlich*, N. Boulis and C. Sahley, Depts of Biology and Psychology, Yale University, New Haven CT. 06511

We have previously shown that the shortening reflex of *Hirudo medicinalis* undergoes two forms of facilitation, dishabituation and sensitization (Boulis and Sahley, 1988). Other labs have suggested a role for serotonin (5HT) in facilitation in *Hirudo* (Berton et al., 1987; Catarsi et al., 1987; Lockery et al., 1987). For this reason, we tested the role of 5HT in facilitation of the shortening reflex. Specifically, we depleted 5HT and observed the subsequent effects on habituation, dishabituation and sensitization.

5HT depletion and histological corroboration were done using a modified version of Lent's technique (1982). Two weeks after the injections half of the leeches were habituated to a low level shock stimulus (2.5-3.5v) applied to the skin of the leech, followed by dishabituation trials using a higher level shock (10V) also to the skin. The remainder of the leeches received sensitization trials using a 10 V stimulus prior to undergoing the habituation regimen.

Glyoxylic acid staining revealed that 5HT was always depleted in the Retzius cells of experimentals. Depletion of the smaller 5HT neurons was variable.

Behaviorally, 5HT depletion attenuated dishabituation and prevented sensitization. In addition, the intial facilitation shown by controls early in habituation training was absent in experimentals.

These results suggest that 5HT, and perhaps Retzius cells play an important role in facilitation of the shortening reflex of *Hirudo*.

ION CHANNELS: CELL FUNCTION

456.1

A DETERMINISTIC MODEL OF ION CHANNELS WITH CHAOTIC BEHAVIOR HAVING A MARKOVIAN-LIKE PROBABILITY FUNCTION. T. I. Tóth* and L. S. Liebovitch* (SPON: A. Sato) Dept. Ophthalmol., Columbia Univ., New York, N.Y., 10032

The most common way to characterize the behavior of ion channels is to analyze the statistical properties of the dwell times in the open and closed states. It is usually assumed that these kinetics are an inherently stochastic process and thus it is characterized by a Markovian stochastic model.

We present a model which is deterministic having chaotic behavior that has statistical properties similar to that of a Markovian stochastic model. This chaotic model is an modified version of a piecewise linear map first used by Lábos to generate neuronal spike-train-like processes. The open and closed time distributions of this chaotic model converge to exponentials similar to that of a two state Markov model. Hence, it cannot be decided alone from the probability distribution of open or closed times whether the underlying behavior of a channel is stochastic or deterministic chaos.

Supported by the American Heart Association, the Whitaker Foundation and NIH EY6234.

456.2

HH CHANNELS ALTER SUMMATION OF SYNAPTIC INPUTS IN A MODEL NEURON *J.W. Moore, M.L. Hines*, & J.K. Gobble**
Dept. Neurobiology, Duke Univ. Med. Cntr. Durham, NC.

We have examined the effect of insertion of Hodgkin-Huxley (HH) channels in passive neuron models of Rall. Computer simulations were carried out on a PC using a new simulation package **CABLE** (Int. J. Biomed. Comp., in press) We have followed, with minor changes, Dodge's (1979, *The Neurosciences, 4th Pgm.*, 439) assignments of morphology and HH channel densities for a motor neuron.

In testing for spike generation by "temporal summation" of two synaptic inputs, we found their **relative timing** to be **more important than their amplitudes** when Na & K channels were present. Contrary to the conventional notion (based on passive membranes and often used in nerve networks) that a spike can be produced by summation of EPSP's to a level exceeding a constant threshold, we find such synaptic inputs will sum to generate a spike only during a relatively brief period when both are rising.

For an "excitatory" synaptic input, the presence of Na channels causes a greater depolarization than in a purely passive neuron. The presence of K channels (with a slowly increasing and decaying conductance) both speeds the return toward rest and reduces the effectiveness of a following EPSP. This inhibitory effect is strong enough to prevent the generation of a spike, even by a train of closely spaced EPSP's.

Conversely, because IPSP's decrease gK and thus make the membrane potential more sensitive to current, they can have an **excitatory** effect for a long period following their peaks.

Neurons with voltage-sensitive channels offer much richer repertoires of integration than do passive membranes.

456.3

A PURKINJE NEURON MODEL WITH ACTIVE DENDRITES. G. T. Bartha* and R. F. Thompson (SPON: W.L.Byerly). Neural, Informational, and Behavioral Sciences, University of Southern California, Los Angeles, CA 90089.

The objectives of detailed modeling of Purkinje cells include reproduction of known physiology to gain insight into underlying mechanisms, exploration of input/output relations to put constraints on possible functions, and integration into a circuit level model.

The simulation code based on the compartmental method allows for the specification of arbitrary cell morphology, distribution of ion channels, and stimuli. The following active ionic conductances have been implemented using Hodgkin-Huxley type equations: 1) the ubiquitous voltage sensitive inactivating Na+, 2) a slower spike-generating Ca++, 3) a voltage sensitive K+, 4) a slow voltage and Ca++ dependent K+, and 5) a non-inactivating Na+ conductance.

Physiological data was better reproduced in Purkinje cell simulations with Na+ currents located in the soma and Ca++ related currents in the dendrite. Several conductance variations showed bursting behavior. Tests of synaptic input/cell firing output gave a near linear relation when somatic conductances dominated dendritic conductances.

Supported by NSF (BNS-8718300), the Mcknight Foundation, and ONR (N00014-88-K-0112) grants to RFT.

456.4

COMPUTATIONAL MODELS OF OLFACTORY RECEPTOR NEURONS: COMPARISON OF CONVENTIONAL AND WHOLE-CELL DATA. F. Pongracz*, S. Firestein and G.M. Shepherd. Section of Neuroanatomy, Yale University Medical School, New Haven, CT. 06510.

Studies of vertebrate olfactory receptor neurons have yielded differing estimates of membrane properties. Compared with conventional intracellular recordings in intact salamander olfactory epithelium (cf. Hedlund et al, J. Neurosci. 7: 2338, 1987), whole cell patch recordings in freshly-dissociated cells show a more negative E_m and a R_N in the Gohm range, implying a correspondingly greater τ_m and dendritic λ (cf. Firestein & Werblin, Proc. Nat. Acad. Sci. 84:6292, 1987).

We have analysed these discrepancies by constructing computational models of the receptor neurons in the two recording situations. The simulations have run on SABER (Analogy, Inc.), a general simulator program that permits rapid exploration of linear and nonlinear parameters.

Analysis of the models has shown that a leak around the electrode, coupled with differences in cell morphology, can account for the lower values of E_m, R_N and τ_m in the conventional recording mode. Peeled exponentials indicate an electrotonic length of the dendrite+cilia of less than 0.3. The results support studies in motoneurons demonstrating the importance of electrode leak in assessing membrane properties. We are extending the models by incorporating transduction-gated conductances in the cilia that are activated by sensory stimulation, and voltage-gated conductances in the soma and axon that generate the impulse response.

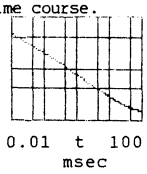
456.5

MODELING INTRACELLULAR ION DIFFUSION. N.T.Carnevale. Neurology Dept. and Dept. of Neurobiology and Behavior, SUNY, Stony Brook, NY 11794.

To study the roles of intracellular ions in neural function, it is necessary to complement experimental observations with quantitative modeling. Electrotonus has been studied extensively, but simulation of diffusion in neurons has received relatively little attention. We have begun to develop guidelines for efficient modeling of diffusion.

One case of particular interest is a cell body of radius r. Assume: rapid Ca influx, so that [Ca] = 1 at time t = 0 in a shell of thickness dr; [Ca] = 0 in the core of the cell at t = 0; there are no binding proteins or pumps. For a cell with r = 20um and dr = 0.1um, Fick's second law predicts that [Ca] in the shell will have many closely spaced eigenvalues (see log-log plot), with the longest tau = 33msec.

The problem is how to design a computationally tractable model to fit such a complex time course. We show how to do this by taking account of the kinetics of Ca currents, Ca-gated conductances, transport mechanisms, and buffers.



456.6

SIMULATION OF PHARMACOLOGICAL RESTORATION OF CONDUCTION FOLLOWING DEMYELINATION. F. N. Quandt and F. A. Davis. Multiple Sclerosis Research Center. Rush University. Chicago, Il. 60612.

Many studies have suggested that the symptoms associated with Multiple Sclerosis are due to conduction block in central nervous system axons secondary to the loss of myelin. It is possible to overcome conduction block following demyelination in an experimental preparation using drugs which increase the duration of the action potential. The agents used to prolong the action potential include K channel blockers and compounds which inhibit Na channel inactivation. The relative effects of the two methods to overcome conduction block on the frequency response of the nerve were determined using a computer model. The method and parameters used for calculating the propagating action potential were similar to those given by Waxman and Wood (*Brain Res.* 294, 1984). In order to study the refractory period, twin current pulses were applied using a variable interstimulus interval. Reductions in the number of myelin wraps in one internodal region led to conduction block beyond the point of demyelination. Either a reduction in g_K or decrease in β_h, the rate of onset of inactivation, restored conduction. However, in the former case a subsequent action potential was blocked beyond the demyelinated internode when the interstimulus interval was short. The conduction velocity increased and the refractory period decreased when either g_K or β_h was reduced. However, for an equivalent change in the conduction velocity, the refractory period was always greater with a reduction in gK. Further, large reductions in g_K increased the refractory period. The results suggest that the demyelinated nerve fiber would have a higher frequency response if conduction is restored by inhibition of Na channel inactivation, compared to K channel block.

456.7

DIFFERENTIAL CONDUCTION IN BRANCHED AXONS: THE ROLE OF THE ELECTROGENIC PUMP. A. Passera* and G. E. Schneider. (SPON: W.A. Rosenblith) Dept. Brain & Cognitive Sciences, M.I.T., Cambridge, MA, 02139.

There has been indirect evidence that differential conduction, which is based on spike frequency, occurs at branch points of particular axons in crayfish and also in leech. However, the crayfish motor system and leech sensory system results appear to reflect different mechanisms. In both systems differential conduction occurs after an increase in threshold following a period of prolonged activity. However, in crayfish the prolonged activity is accompanied by a slight depolarization, while in leech prolonged activity is accompanied by hyperpolarization. In addition, artificial hyperpolarization relieves the conduction failure in crayfish, but mimics conduction failure in leech. Furthermore, inactivating the electrogenic pump enhances conduction failure in the crayfish preparation, but relieves conduction failure in the leech preparation.

A computer simulation has been used to explore the hypothesis that both differential conduction and the differences in excitability changes in the two preparations can be explained by different axon-specific densities of electrogenic pump sites. The mechanism to account for differential conduction involves sensitivity of the electrogenic pump to external potassium and internal sodium concentrations, which causes the pump to activate sooner in a thin branch than in a thicker branch. Consequently, there can be differences in excitability in two branches of the same axon, even though the density of pump sites per unit area of membrane is uniform. The differences in equilibrium potential accompanying prolonged activity in the two preparations can be accounted for by different overall densities of pump sites in the leech and the crayfish preparations with the density in the leech preparation being much higher.

Supported by NIH grant 00126, NIGMS T32-GM07484, & Interactions Co., Cambridge, Ma.

456.8

ACTION POTENTIAL DURATION IN A RAT PITUITARY CELL LINE (GH3) IS DEPENDENT ON INTRACELLULAR CALCIUM ACCUMULATION. S. M. Simasko. Dept. of Physiology, State Univ. of New York at Buffalo, Buffalo, NY 14214.

Because the amount of calcium that would enter a GH3 cell through voltage-dependent calcium channels is dependent on action potential duration, experiments were performed to determine what factors regulate this duration. Action potentials were measured using the whole-cell variation of the patch-clamp technique. The effect of accumulation of free intracellular calcium on action potential duration was examined by manipulation of either the bath calcium concentration or the calcium buffering capacity of the pipette buffer. Under standard conditions (bath: 2 mM calcium; pipette: 2 mM EGTA/100 nM free calcium) action potential duration was found to be 240 ± 64 msec. When pipette calcium buffering capacity was reduced (0.2 mM EGTA/100 nM free calcium) action potential duration decreased to 79 ± 7 msec. When pipette calcium buffering capacity was increased (2 mM EGTA/no added calcium) action potential duration increased to 1880 ± 715 msec. When bath calcium was increased to 10 mM with either standard or reduced pipette calcium buffering capacity, action potential duration decreased (57 ± 4 % and 36 ± 3 % of control, respectively). These results indicate that action potential duration is determined by the speed at which intracellular free calcium accumulates. This would imply that without a change in the calcium sensitivity of the event that mediates repolarization (activation of a potassium channel or inactivation of the calcium current) any increase of intracellular calcium during a single action potential is self-limiting.

456.9

IONIC CURRENTS IN RAT LACTOTROPHS. D. Janigro, G. Maccaferri* and J. Meldolesi*. Dept. of Pharmacology, H San Raffaele 20132 Milan Italy.

Ionic currents were investigated in purified rat lactotrophs in primary culture. Patch clamp experiments using the whole cell configuration mode revealed large potassium currents elicited from a holding potential of -110 mV when recordings were performed in standard extracellular solution (in mM: 150 NaCl, 5 KCl, 2 $CaCl_2$, 2 $MgCl_2$, 5 HEPES, 5 Glucose). An early outward current required calcium influx for its activation since removal of extracellular calcium ions abolished this component thus permitting the study of a long lasting current. After blockade of potassium channels with intracellular CsCl (130 mM) and external TEA (130 mM), low and high threshold calcium currents were elicited from a holding potential of -100 mV and -40 mV, respectively. Partial inhibition of the inward currents was obtained after cell exposure to amiloride (250 uM) and nitrendipine (10 uM).

These results confirm the existence of multiple calcium and potassium conductances in rat lactotrophs.

456.10

SODIUM CONDUCTANCE GENERATES THETA RHYTHMICITY IN ENTORHINAL CORTEX LAYER II STELLATE CELLS *IN VITRO*. A. Alonso* and R. Llinás (SPON: J. Ransohoff). Dept. Physiol. & Biophys., New York Univ. Med. Ctr., New York, NY 10016.

Stellate cells from the entorhinal cortex (EC) layer II form part of the network that generates the theta rhythm and give rise to the most prominent component of the perforant path. Intracellular recordings were obtained from EC layer II stellate cells in rat brain slices. The neurons were identified on the basis of their location and, following biocytin injections, by their morphology. These cells have an input resistance of 25.3 ± 9.5MΩ (n=20) and a resting potential of -64.2 ± 3.6 mV and demonstrate pronounced delayed and anomalous rectifications. In addition to fast action potentials, the stellate cells showed rhythmic subthreshold oscillatory activity. Depolarizing constant current injection was accompained by rhythmic voltage oscillations having a sinusoidal-like appearence and a frequency which ranged from 5 to 11Hz. This oscillatory activity developed and reached rather maximum amplitude at a membrane potential of -59 to -54mV. Bath application of TTX, replacement of extracellular sodium by choline, and intracellular inyection of QX314, always resulted in a complete abolition of the oscillations, which persisted after calcium channels blockage. These results indicate that in the EC stellate cells the subthreshold oscillatory events observed upon depolarization are driven by a persistent voltage-dependant sodium conductance. Voltage-clamp analysis confirmed the presence of such current and revealed that the hyperpolarizing phase of the oscillation is due to time-dependant inactivation of the sodium current and to the delayed activation of an outward current. The finding described above indicates that the EC layer II have an intrinsic subthreshold oscillatory propertie that differs from those previously described in the CNS. The results are significant as they indicate that an invariant rhythmicicty in the brain (in this case the limbic theta rhythm) does not require a common ionic mechanism for the different cell groups involved in the particular network demonstrating the macroscopic rhythmicity.

456.11

SUBTHRESHOLD MEMBRANE POTENTIAL OSCILLATIONS MODULATE REPETITIVE FIRING OF MESOPONTINE CHOLINERGIC NEURONS *IN VITRO*. C.S. Leonard and R. Llinás, Dept. Physiol. & Biophys., NYU Med. Ctr. 550 First Ave. New York, NY 10016

The mechanisms underlying repetitive firing of cholinergic pedunculopontine and laterodorsal tegmental neurons were investigated by intracellular recording in guinea pig brain slices. Cholinergic neurons were identified physiologically and morphologically by combined intracellular injection of lucifer yellow and histochemical staining for NADPH-diaphorase (Leonard and Llinás, '88, Soc. Neurosci. Abst. 14: 297), which is a selective label for mesopontine cholinergic neurons (Vincent et al., '83, Neurosci. Letts. 43: 31-36). At just subthreshold potentials (-50mV), spontaneous 10Hz membrane potential oscillations were observed to underlie repetitive firing. The 10Hz oscillation was sustained for 3-8 cycles before breaking into oscillations composed of several frequencies (5-15Hz). Oscillations occurred at potentials between spike threshold and -60mV, were maximal (2-5mV) near threshold and were reduced in amplitude by membrane hyperpolarization. Blockage of high threshold Ca spiking and synaptic transmission with low- Ca/ 2mM Co or normal Ca/ 0.2mM Cd Ringer did not abolish the oscillations. However, they were completely abolished in Ringer containing TTX (1µg/ml). These observations imply that the oscillations are intrinsic and arise through activation of a persistent Na conductance. The intrinsic nature of the oscillations was also indicated by the fact that transient oscillations were evoked by brief (2ms) depolarizing current pulses from hyperpolarized membrane potentials. Furthermore, small hyperpolarizing pulses (<5mV, 2ms) were sufficient to reset the phase of spontaneous oscillations. Functionally, action potentials arise from the depolarizing phase of these membrane oscillations and thus, the temporal pattern of action potentials is established by the interplay between oscillations and the Ca- dependent post-spike hyperpolarizations also present in mesopontine cholinergic neurons. Membrane oscillations may also have important consequences for controlling the relative synchrony of these neurons, since a common, transient input to the population would reset the oscillations and, at least temporarily, promote phase-locked firing. Support NINCDS13742.

456.12

Na^+ AND Ca^{2+} VOLTAGE-DEPENDENT CHANNELS ARE INVOLVED IN PLATELET ACTIVITY. A. Gual*, L. Palacios-Araus* and J. Palés* (SPON: C. González). Lab. of Neurophysiology and Biomembranes, Faculty of Medicine, University of Barcelona, 08028 Barcelona, SPAIN.

The role of membrane potential and the involvement of voltage-dependent mechanisms in the stimulus-response coupling in platelets are controversial. Na^+ and Ca^{2+} fluxes seem to be involved in platelet activity (Wencel-Drake, J.D. and Feinberg, H., Thromb.Haemost., 53:75, 1985). We have therefore studied the effects of extracellular Na^+ and tetrodotoxin (TTX), and dihydropiridines (DPH) on platelet aggregation and platelet cytosolic free Ca^{2+} in normal and depolarized platelets. In control platelets, zero extracellular Na^+ or 3×10^{-8}M TTX block aggregation and inhibit the concomitant rise of cytosolic free Ca^{2+} induced by thrombin. Both manoeuvers fail to inhibit aggregation in high-K^+ depolarized platelets. In control platelets, 50×10^{-9}M nisoldipine blocks both platelet aggregation and the concomitant cytosolic free Ca^{2+} rise induced by thrombin; these effects were enhanced in K^+-depolarized platelets. These results indicate that the platelet activation is sensitive to extracellular Na^+, TTX and DHP, and suggest that platelet activity could be voltage-modulated. Supported by grant 87-1346 FISss, Spain.

456.13

A MODEL OF EXOCYTOSIS BASED ON THE DEPLETION OF IONS FROM THE SPACE BETWEEN VESICLE AND PLASMA MEMBRANES. G. Ehrenstein* and E.F. Stanley (Spons: K. Krebs) LB, NINDS, NIH, Bethesda, MD 20892.

We propose the following revised version of a model for the mechanism for calcium-dependent exocytosis: Increased cytosolic calcium concentration causes opening of a calcium-activated potassium channel located in the secretory vesicle opposite the plasma membrane. Opening of the channel causes influx of potassium ions into the vesicle. This tends to deplete salt concentration in the space between the vesicle membrane and the plasma membrane. In presence of calcium, this space is extremely narrow, limiting replacement of lost ions. Because of net ion loss, the space becomes hypoosmotic and water leaves, causing the two membranes to fuse.

Because of the small size of the space which becomes hypoosmotic, only a very small number of molecules leave. Therefore, it may be possible for sodium or sugar molecules to take the place of potassium in lowering the osmolarity of the space. Thus, the experimental evidence that replacement of cytosolic potassium by sodium or by sugar has no effect on the rate of exocytosis, does not necessarily contradict the model.

456.14

RELATIONSHIP BETWEEN HORMONE SECRETION AND CALCIUM CHANNEL ACTIVITY IN ANTERIOR PITUITARY CELLS. Miquel Puigmacia* & Susan A. DeRiemer. (SPON: C. Scheffey), Columbia University, New York, NY. 10027

Secretion in pituitary cells is dependent on calcium influx via voltage sensitive ion channels or release from intracellular stores. In order to understand the relationship between calcium influx and secretion we have characterized the actions of different calcium channel types in cultured rat anterior pituitary cells by measuring hormone secretion in isolated cells with the reverse hemolytic plaque assay and by release experiments on cell populations by RIA. In previous electrophysiological studies it was found that somatotrophs (GH cells) have only L type calcium channels (no T or N), while lactotrophs (PRL) have both T and L (no N). ω-Conotoxin, an L blocker, produced a clear decrease in the percentage of GH cells secreting and in the diameters of plaques. A similar, but less pronounced, effect was observed in PRL cells. Bay K8644, the dihydropyridine L channel agonist, caused a significant increase in hormone secretion from both cell types, but again was less potent in the PRL cells. The inorganic calcium channel blockers Cd^{2+}, Co^{2+}, and Gd^{3+} were also tested. The first two produced complete block of secretion at doses under 100 µM in both cell types. Preliminary evidence suggests that Gd^{3+} may also inhibit secretion despite the absence of N type channels. Alcohols had no effect on GH cells.

456.15

N-TYPE CA2+ CHANNEL-DEPENDENT AND INDEPENDENT RELEASE OF 3H-DOPAMINE FROM CULTURED MESENCEPHALIC NEURONS. M.G. Grilli, A.G. Wright, Jr.* and I. Hanbauer. H-E Branch, NHLBI, N.I.H., Bethesda, MD 20892.

Mechanisms involved in the release of ^{3}H-dopamine (^{3}H-DA) elicited by different membrane depolarizing agents were studied in primary cultures of dopaminergic neurons. Dissociated neurons of ventral mesencephalon from fetal rats grown in culture for five days were preloaded with ^{3}H-DA (5×10^{-8}M) and the basal ^{3}H-DA release in standard Krebs-Ringer-Henseleit buffer was determined. K^+ elicited a concentration-dependent increase of ^{3}H-DA release (25 mM K^+ elicited half maximal increase) that was blocked when cell cultures were preincubated with 10^{-5} M omega-conotoxin. The K^+-elicited increase of ^{3}H-DA release was not altered by nifedipine, amiloride or an endogenous Ca^{2+} channel modulator that was shown to block L- and T-type channels (Callewaert et al., Science 243:663, 1989). Veratridine also caused a concentration-dependent increase of ^{3}H-DA release (2×10^{-6}M veratridine elicited half maximal increase) that was tetrodotoxin sensitive, but was not inhibited by 10^{-5} M omega-conotoxin. Moreover, nifedipine, amiloride, or the endogenous Ca^{2+} channel modulator, failed to prevent the veratridine-stimulated ^{3}H-DA release. The present results show that ^{3}H-DA release elicited by K^+ involves the activation of the N-type Ca^{2+} channel while ^{3}H-DA release elicited by veratridine is independent of Ca^{2+} influx through voltage-gated Ca^{2+} channels.

456.16

ANTI-CALMODULIN AGENTS (ACA) AFFECT DEPOLARIZATION- AND CALCIUM IONOPHORE-INDUCED VASOPRESSIN (AVP) RELEASE BY AN ALTERNATIVE MECHANISM. N. F. Rossi. Dept. of Medicine, Wayne State Univ. School of Medicine, Detroit, MI 48201.

Calmodulin has been implicated in transducing the effects of calcium on synaptic transmission and hormone release, including osmotically-stimulated AVP release. If ACA block AVP release secondary to inhibition of calcium-calmodulin interactions, these drugs should inhibit AVP release to stimuli increasing calcium influx via different mechanisms. Hypothalamo-neurohypophysial complexes (HNC) in culture were exposed to 2µM ionomycin, 1µM Bay k 8644, or 75µM veratridine either alone; with an ACA (1µM TFP, 1µm W7, or 2µM R24571); or with calcium channel blocker (0.5µM D600). Ionomycin produced an 593±97% increase in AVP release compared with basal release ($p<0.001$, n=14). All the ACA significantly decreased the response to ionomycin (173±54%, 192±30%, 137±57% of basal AVP; n=7, 6, 8; $p<0.05$ vs ionomycin) while D600 did not. Bay k 8644 stimulated AVP to 499±74% of basal ($p<0.005$, n=8). None of the ACA inhibited this response, but D600 completely blocked it (66±17% of basal; n=6; $p<0.01$ vs Bay k 8644). Neither ACA nor D600 prevented the rise in AVP with veratridine (419±59% of basal; n=14; $p<0.001$ vs basal). These results are consistent with the hypothesis that ACA inhibit AVP release by membrane stabilizing effects rather than by antagonizing calcium-calmodulin in HNC. Depolarization initiated by sodium influx may stimulate sodium-calcium exchange independent of slow calcium channels.

456.17

MECHANISMS OF ANOXIA TOLERANCE IN THE TURTLE BRAIN. C.J Doll*, R.K. Suarez*, P.W. Hochachka* and P.B. Reiner (SPON: D. Schwarz). Depts. of Zoology and Psychiatry, Univ. of British Columbia, Vancouver, BC, Canada.

The brain of the turtle *Chrysemys picta* exhibits remarkable anoxia tolerance. One explanation for this phenomenon might be that turtles have low metabolic rates both during normoxia and anoxia, allowing glycolytic ATP production to satisfy neuronal energy requirements. Normoxic metabolic rate was measured using the [^{3}H] 2-deoxyglucose method *in vivo*. The turtle brain consumed 0.053 μM/g/min at 25°C, 1/12 the published glucose uptake rates for rat brain. Rat brain homogenates exhibited a 2-fold greater Na^+,K^+-ATPase activity on average as compared to the turtle brain at the same temperature. These data suggest that ion leakage and pumping in the normoxic turtle brain is less than that in the rat brain. To obtain a measure of ion leakage, intracellular recordings of cortical pyramidal neurons *in vitro* were performed during normoxia and anoxia. Time constants, which reflect the product of membrane resistance and capacitance, did not increase with anoxia, but were significantly greater in turtle than rat, suggesting lower normoxic ion fluxes in the turtle brain. In contrast to the results with time constants, apparent input resistance decreased with anoxia. Current efforts are directed towards determining whether increased synaptic activity is responsible for this change.

456.18

THE ROLE OF CALCIUM IN ANOXIC INJURY IN MAMMALIAN CENTRAL WHITE MATTER. P.K.Stys, B.R.Ransom,S.G.Waxman. Dept. of Neurology, Yale Univ., New Haven, CT 06510.

The effects of anoxia on the CNS have been extensively studied in gray matter, but much less is known about the consequences of oxygen deprivation in white matter. We have used the isolated rat optic nerve (RON), a typical central white matter tract, to study the pathophysiology of anoxic injury in mammalian white matter (Davis and Ransom,Soc.Neurosci.Abstr.,13:1634,1987). The area under the compound action potential, recorded with suction electrodes, was used as an indicator of functional integrity of the RON, allowing quantitative evaluation of recovery after anoxic insults and of the effects of various interventions. RONs from adult (50-70 days) animals were dissected free, placed in a perfusion chamber and subjected to a standard 60 minute period of anoxia in a N2/CO2 atmosphere. We confirmed previous findings of 20-30% recovery after anoxia (as judged by the area under the compound action potential) when the preparation was perfused with standard solution containing 2mM Ca2+; reduction of external Ca2+ to 0 mM greatly improved recovery (>80%). On the assumption that Ca2+ flux across the membrane is a critical factor responsible for irreversible anoxic injury, an attempt was made to interfere with its presumed entry through conventional channels. Pre-treatment with nifedipine (1-10μM), an organic Ca2+ channel blocker, did not significantly improve recovery. Likewise, divalent cations such as Co2+ (1-4 mM) and Zn2+ (0.1-1mM) were without benefit and, paradoxically, at the higher concentrations resulted in a complete and irreversible loss of activity after anoxia. However, high concentrations of Mg2+ (10mM) significantly improved recovery (mean 64%, $p<0.05$). We conclude that disordered regulation of Ca2+ homeostasis induced by anoxia plays a central role in producing irreversible dysfunction in the RON. Furthermore, redistribution of Ca2+ under these conditions may not utilize conventional channels, since known blocking agents conferred no increased resistance to anoxia.

Supported by NIH and VA grants; P.Stys is a BVA fellow.

456.19

VOLTAGE- AND LIGAND-GATED CURRENTS IN CULTURED OLFACTORY INTERNEURONS FROM AN INSECT BRAIN. J.H. Hayashi and J.G. Hildebrand. ARL Div. of Neurobiol., Univ. Arizona, Tucson, AZ 85721.

We are examining the voltage- and ligand-gated conductances of solitary neurons in primary cell cultures of dissociated neurons from antennal lobes (ALs -- the primary olfactory centers in the brain) of the moth *Manduca sexta* as part of our ongoing studies of mechanisms that underlie the central processing of olfactory information. There are two basic classes of neurons within the AL: local interneurons (LNs) and projection (output) neurons (PNs). In cultures derived from the medial group of AL neurons, consisting exclusively of PNs, we previously identified 6 different morphological types [*Soc Neurosci Abs* 14:380 (1988)]. We have now extended our analysis to cultures derived from the lateral group of AL neurons that consists of both PNs and LNs. Along with the previously reported PN cell types, we have found 3 new types of neurons in these culture. Cells of 2 of these new types clearly lack an axonal process. These neurons exhibit vigorous process outgrowth, but our patch-clamp studies have revealed that they fail to develop voltage-gated inward currents after 2 weeks *in vitro*. In contrast, all PNs develop inward currents after a comparable time in culture. We have also extended our analysis of PNs. A subset of PNs expresses tetrodotoxin-sensitive Na^+ channels. This current activates at about -40 mV and is associated with a delayed-rectifier type of K^+ channel. We also find slowly activating, slowly inactivating Ca^{++} channels that are blocked by Cd^{++}. "Whole-terminal" recordings have permitted accurate measurements of currents in the terminal region. Each type of PN displays a characteristic set of currents, and some exhibit an outward current in response to a puff of GABA solution applied externally. GABA is the putative neurotransmitter used by most of the LNs. [Supported by a grant from Monsanto Company and an NIH postdoctoral fellowship (to JHH), and in part by NIH grant AI-23253.]

456.20

The kinetics of M-currents (I_M) recorded in bullfrog sympathetic ganglion neurons. M.E.M. KELLY AND P.S. PENNEFATHER. Faculty of Pharmacy, University of Toronto, Toronto, Ontario, CANADA M5S 2S2.

In bullfrog sympathetic cells inhibition of a voltage-dependent K^+ current, I_M, by muscarinic agonists results in depolarization. I_M begins to activate at -60 mV and is fully activated at -20 mV, playing an important role in spike frequency adaption. The time dependence of I_M has been reported to be adequately accounted for by a two state kinetic model where the rates of channel opening and closing are voltage sensitive to an equal degree. (Adams et al., 1982, J. Physiol, 330, 537). That model predicts significant activation of I_M by individual action potentials. We have found, however, that brief action potential-like voltage-clamp commands activate much less I_M than predicted. We recorded I_M in acutely dissociated ganglion neurons using whole cell recording and discontinuous single electrode voltage-clamp. Under conditions where currents other than I_M were suppressed, I_M was defined as the difference between currents recorded in the presence and absence of muscarine. This allowed us to study I_M kinetics over a wide range of membrane potentials. We found significant delays in I_M activation lasting 4-10 ms depending on command potential. These delays are suggestive of multiple closed-states and ensure that I_M depends on mean membrane potential rather than action potential frequency.

456.21

WITHDRAWN

456.22

MEMBRANE IONIC CURRENTS IN IDENTIFIED NEURONS OF A JELLYFISH. J. Przysiezniak and A. N. Spencer. Dept. of Zool., Univ. of Alberta, Edmonton, AB, CANADA, T6G 2E9 and Bamfield Marine Station, Bamfield, BC, CANADA, V0R 1B0.

Nervous systems first evolved in the Cnidaria. We have described several neuronal membrane ionic currents in a hydrozoan jellyfish, *Polyorchis penicillatus* to determine whether they differ substantially from those observed in 'higher' phyla and in Protozoa.

It is possible to isolate and culture a subset of large, identifiable motor neurons from *Polyorchis*. *In vitro*, 'whole-cell', current-clamp recordings show that these cells produce plateau action-potentials of variable duration, as they do *in vivo*. Voltage-clamp data have revealed that at least five distinct currents are at play. A large (~5nA), rapidly activating, transient, inward sodium current produces the upstroke of the action potential; this current is TTX-insensitive. A small (500pA), rapidly activating, transient (τ ~20ms) calcium current contributes to the rising phase, and together with a slow, sustained calcium current, underlies a slowly repolarizing plateau phase. A large (~3nA), sustained, potassium mediated, delayed rectifier contributes to spike repolarization. Spike repolarization is also effected by a large (~5nA) but transient (τ-off ~200ms), 'A-like' outward current which inactivates at depolarized voltages, thus regulating action-potential duration in a voltage-dependent manner.

It appears that the complement of membrane currents found in these primitive neurons resembles that seen in 'higher' phyla, and differs in quality, but not in diversity, from that observed in *Paramecium*.

456.23

VOLTAGE CLAMP ANALYSIS OF IONIC CONDUCTANCES IN TWO TYPES OF SENSORY NEURONS IN THE LEECH J. Johansen and A.L. Kleinhaus. Dept. of Zoology, Iowa State Univ., Ames, IA 50011 and Dept. of Neurology, Yale Univ. School of Medicine, New Haven, CT 06512.

With the ultimate goal of correlating the biological function of neurons with their biophysical properties we have begun a systematic analysis of the ionic conductances underlying excitation in the lateral nociceptive (N_l) and medial pressure (P_m) cells in the leech *Macrobdella*. The two cells differ in their functional sensory modalities and in their excitable properties. Using two electrode voltage clamp techniques we find that both N_l and P_m possess an I_{Na}, I_K, and I_{Ca}. The peak Na-conductance is for voltage steps from resting membrane potential (-40 mV) to 10 mV. At this membrane potential at 10 °C I_{Na} activates with a time constant of 1.6 ms and inactivates with a time constant of 9 ms. The kinetics of I_{Na} is best described by a Hodgkin-Huxley equation of the form $I_{Na}=g_{Na}m^4h$. The delayed rectifier current (I_K) activates with time constants of from 6 to 15 ms at 22 °C in response to membrane steps to between -15 mV and 15 mV. This current was sensitive to TEA. I_{Ca} does not inactivate appreciably during long voltage steps (>500 ms). The kinetic properties of these conductances were similar in both cell types. However, in addition to these currents N_l possesses an I_A-current with rapid activation and inactivation characteristics. In contrast, we have not yet been able to identify an I_A-current in the P_m cell suggesting a possible mechanism for the differences in the two cells' excitability. This possibility and a further characterization of the two cells' conductances are currently being investigated.

456.24

WHOLE CELL PATCH CLAMP RECORDINGS OF VOLTAGE-DEPENDENT CURRENTS IN THIN MEDULLA SLICE INCLUDING IDENTIFIED TRIGEMINOTHALAMIC NEURONS. L.Chen and L.-Y.M. Huang. Marine Biomedical Institute, University of Texas Medical Branch, Galveston, TX 77550.

In order to characterize the synaptic transmission to the trigeminothalamic neurons, we examined the electrical properties of neurons in caudal spinal trigeminal nucleus. Trigeminothalamic neurons were labeled with retrograde neuronal marker fluorescent latex microspheres. Thin slices were prepared from the lower medulla of the injected rats using the method described by Edwards, Konnerth, Sakmann & Takahashi (Pflugers Archiv, in press). The surface of the recording cells was blown clean with buffer, and whole cell recordings were then performed. Under voltage clamp conditions, both inward and outward currents were activated when a series of depolarizing pulses ranging from -60 to +150 mV were applied to the cell. Inward currents were examined by replacing K^+ with Cs^+ or N-methyl-D-glutamine intracellularly, and by treating cells with tetraethylammonium (TEA) and 4-aminopyridine. Most inward current could be eliminated by low concentration of tetrodotoxin (TTX). Na^+ was the principal ion carrier of the inward current. In the presence of 1 μM TTX and 5-10 mM Ca, slow inward current was observed. This current was sensitive to external Ca concentration and was abolished by Co^{++}. Voltage-dependent outward currents were carried by K^+ and were blockable by TEA. The voltage properties of these currents were similar to those observed in isolated dorsal horn projection neurons (J. Physiol. 411:161-177, 1989). Supported by NS23061 and NS01050.

456.25

EXPRESSION OF AMINO ACID TASTE RECEPTORS IN *XENOPUS* OOCYTES.
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Roche Inst. Molec Biol., Nutley, N.J.07110 and Monell Center, Phila. PA 19104

We report the first *in vitro* demonstration of functional expression of vertebrate taste receptor molecules. Isolation, characterization and cloning of the molecules responsible for the primary binding event in vertebrate chemoreception is dependent on functional *in vitro* assays encompassing the full range of transductive events from the initial ligand binding through changes in gated membrane conductances. Expression in the *Xenopus* oocyte has been used to identify, functionally characterize and clone receptors for many bioactive compounds. Getchell (1988) reported that *Xenopus* oocytes microinjected with rat olfactory RNA express functional olfactory receptor molecules as monitored electrophysiologically. We now show the ability of *Xenopus* oocytes microinjected with catfish barbel mRNA to express functional amino acid taste receptors. Electrophysiological recordings from these injected oocytes demonstrate the expression of amino acid specific conductance changes analogous to those in intact catfish. Injected oocytes manifest distinct and reproducible time-conductance profiles following stimulation with L-arginine or L-alanine both of which are taste stimuli in catfish. The responses exhibit molecular specificity, reproducibility, rapid onset and short duration. Repetitive stimulation at short intervals or prolonged stimulation results in adaptation that is reversible with full return of response capabilities. Use of brief pulses of the stimulus ligand prevented receptor desensitization enabling us to demonstrate the expression of taste receptors in *Xenopus* oocytes. This defined *in vitro* system permits physiological, biochemical and molecular biological studies of taste receptors. NIH-NS16340&F33NS08486(TVG).

456.26

COMPARISON OF ENDOGENOUS AND EXOGENOUS EXPRESSION OF ELECTRICAL COUPLING IN PAIRED XENOPUS OOCYTES.
L.C. Barrio*, J.C. Saez*, C. Samathanam*, R.S. Zukin and M.V.L. Bennett. Dept. Neurosci., A. Einstein Coll. Med., Bronx, NY 10461.

Xenopus oocytes (stage VI) were defolliculated and injected with rat brain, hippocampal or liver poly $(A)^+$ RNA or with water. After 1-3 days (to allow for protein synthesis) vitelline membranes were removed and pairs of cells placed in contact. One or more days later junctional conductance (g_j) at low transjunctional voltages (g_{max}) and voltage dependence of g_j were measured by dual voltage clamp. In water-injected (control) oocytes g_{max} ranged from <0.01 μs to 7.5 μs (n >30). In mRNA-injected oocytes g_{max} was as large as 12.5 μs but overlapped with values for control oocytes. Pairs injected with brain mRNA showed punctate immunoreactivity between them to a connexin 43 antibody, perhaps from expression of astrocyte mRNA. Cycloheximide (50 μg/ml) (but not actinomycin) prevented coupling of control oocytes. Thus, post pairing translation contributed to endogenous coupling. Junctions between most oocytes were voltage dependent. In almost all control oocytes the degree of voltage dependence was less than in amphibian blastomeres. Since transcription should be minimal in the period between oocyte maturation and gastrulation, the endogenous mRNA may selectively express a different connexin than do blastomeres, or there may be posttranslational modification that reduces voltage dependence. In liver-injected pairs voltage dependence must have arisen in channels with low sensitivity like those observed in hepatocytes. In brain-injected pairs voltage dependence could be accounted for by voltage dependent endogenous channels and voltage independent exogenous channels in parallel. Some junctions rectified, and in pairs injected with brain mRNA in only one cell, conductance decreased with the endogenous side positive as predicted from previous work. The data indicate that endogenous and exogenous expression occur and may interact.

456.27

FLUORESCENCE RECORDING OF ACTION POTENTIALS USING A SINGLE OPTICAL FIBER CARRYING EXCITATION AND FLUORESCENT LIGHT. V Krauthamer*, TA Bowmaster*, CC Davis*, and HJ Bryant, FDA Center for Devices and Radiological Health, Rockville, MD 20857, and USUHS, Dept. of Physiology, Bethesda, MD 20814.

Fluorescent dyes have been used previously to record voltage changes in a variety of preparations. Existing systems generally use separate optical paths for excitation and detection of the fluorescent signal or employ a microscope objective or other large lens system near the preparation which limits their use in confined areas. We used a single optical fiber to both excite and detect voltage-sensitive fluorescence signals from frog myocardium stained with WW781. Light from a He-Ne laser, after passing through a neutral density filter and a beam splitter, was coupled to an optical fiber with an inner light-carrying core diameter of 4, 50, or 100 μ. The various fibers used had outside diameters of 120 μ due to optical cladding. The longer-wavelength fluorescent light from the preparation was collected by the same fiber and reflected, through a blocking filter, into either a photomultiplier tube or a photodiode. Frog hearts, maintained in a 0 Ca^{++} solution to prevent movement artifacts, produced a fractional change in fluorescence of ~10^{-3} during the myocardial action potential. Large-core fibers yielded quieter signals, which allowed for reduced excitation light intensity with less dye bleaching, but at the expense of the higher spatial resolution obtained with the single mode 4 μ fiber.

457.1

SELECTIVE ACTIVATION OF Ca++ ENTRANCE THROUGH VOLTAGE-OPERATED CHANNELS OR THROUGH THE Na+-Ca++ ANTIPORTER IN RAT BRAIN SYNAPTOSOMES: METHODOLOGICAL AND PHARMACOLOGICAL INDICATIONS. M.Taglialatela*, L.Canzoniero*, A.Fatatis*, S.Amoroso*, G.F.Di Renzo* and L.Annunziato§. Ist. Pharmacology, 2nd Sc.Med., University of Naples,Via Pansini 5,80131 Naples,and Univ.of Chieti(§),Italy.

Ca++ entrance in nerve cells can also occur either through Voltage-Operated Ca++ Channels (VOCCs) or through the membrane Na+-Ca++ antiporter, which couples the bi-directional transport of Ca++ ions to that of Na+ ions in the opposite direction. The aim of the present study was to identify the appropriate conditions which selectively activate only one of the two pathways mediating 45Ca++influx in rat brain synaptosomes. Increasing extracellular concentrations of K+ ions caused: 1.a progressive depolarization of the synaptosomal membrane (5-70mM K+ ions), estimated by the potential-sensitive fluorescent dye bisoxonol, 2.a dose-dependent increase in 45Ca++ uptake, 3. a rapid and dose-dependent (10-30 mM extracellular K+ ions) increase in cytosolic Ca++levels monitored by FURA-2.45Ca++ uptake elicited by concentrations of K^+ ions up to 55 mM seems to occur selectively through VOCCs, since an equimolar concentration of choline,in presence of the same extracellular Na+ concentration (95 mM), failed to stimulate 45Ca++ uptake.A further decrease of extracellular Na+ concentrations from 95 mM to 0 (145 mM choline substitution),dose-dependently increased 45Ca++ uptake via the Na+-Ca++ antiporter,without any concomitant activation of VOCCs, since, under these conditions, membrane potential did not show any tendency to depolarization.Furthermore,the amiloride analogue 2',4'-dimethylbenzamil amiloride(2',4'-DMB,10-300 uM),a selective inhibitor of the Na+-Ca++ exchanger,inhibited 45Ca++ uptake elicited by extracellular Na+ ions removal, whereas it did not interfere with 55 mM K+-induced 45Ca++ uptake in purified synaptosomes.

457.2

CYTOSOLIC Ca++ LEVELS AND MEMBRANE POTENTIAL MONITORING IN BRAIN SYNAPTOSOMES: EFFECT OF MARINE TOXINS WITH AGONISTIC AND ANTAGONISTIC PROPERTIES ON Ca++ CHANNELS. L.Canzoniero*,M.Taglialatela*,A.Fatatis*,S.Amoroso*,G.F.Di Renzo* and L.Annunziato§ (SPON: A.Volterra). Ist.Pharmacology, 2nd Sc.Med., Univ.Naples, Via Pansini 5, 80131 Naples, and Univ.Chieti(§), Italy.

Toxins that act on ion channels have proven to be invaluable tools in the biochemical and functional characterization of these membrane proteins. The aim of the present study was to clarify the effects of maitotoxin (MTX), a marine toxin which has recently gained attention as a putative Ca++ channel activator, on cytosolic Ca++ levels and membrane potential variations in purified rat brain synaptosomes. MTX (3-25 ng/ml), dose-dependently enhanced cytosolic Ca++ levels, monitored with the Ca++-sensitive fluorescent dye FURA-2. This effect was prevented when Ca++ ions (plus 1 mM EGTA) were omitted from the extracellular medium. Furthermore, omega-conotoxin (wCTX), the recently described presynaptic neurotoxin provided with antagonistic properties on Ca++ channels,was unable (1-10 uM) to prevent the increase of cytosolic Ca++ levels induced by MTX.On the other hand,MTX (10 ng/ml) caused an intense depolarization of the synaptosomal membrane, estimated by means of the potential-sensitive fluorescent dye bisoxonol. This increase in membrane depolarization was unaffected by tetrodotoxin (TTX, 10 uM), and only slightly prevented by the replacement of extracellular Na+ions with an equimolar concentration of choline. By contrast, when sucrose replaced isoosmotically monovalent cations, MTX was unable to induce any change in the synaptosomal membrane potential.
Collectively, these results appear to suggest that MTX-induced stimulation of extracellular Ca++ influx can occur as a consequence of the depolarizing effect exerted by the marine toxin on the synaptosomal membrane.

457.3

INFLUENCE OF PERTUSSIS TOXIN PRETREATMENT ON DIHYDROPIRIDINE MODIFICATION OF K^+ AND MAITOTOXIN INDUCED INTRACELLULAR CALCIUM RISE IN PC12 CELLS. G.Schettini, O.Meucci*, M.Grimaldi* T.Florio*, E.Landolfi*, A.Scorziello*, C.Ventra* and A. Marino*. Inst. of Pharmacology, II School of Medicine Univ. of Naples, Via S.Pansini 5, 80131 Naples ITALY(SPON:U.DiPorzio)

Here we report the result of pertussis toxin (PTX) pretreatment on dihydropiridine inhibition of free intracellular Ca^{++}increase induced by both K^+depolarization and maitotoxin (MTX), a marine toxin known to increase cytosolic Ca^{++} levels, in PC12 cells. Nicardipine (Nic) 100 nM reduced K^+ induced Ca^{++}rise to 25%, while a complete inhibition of K^+ effect was obtained for Nic micromolar concentrations. Nic (from 10nM to 1uM) dose-dependently inhibited MTX-induced Ca^{++}increase with a maximal inhibiton (-87%) for Nic 1uM. Furthermore, the dihydropiridine derivative Bay K 8644 enhanced K^+evoked Ca^{++}rise in a dose dependent manner,an effect antagonized by equimolar concentrations of Nic. Finally pretreatment with PTX partially but significantly reverted Nic inhibition of MTX stimulation for all Nic concentrations tested (-58% in PTX vs.-87% in control, for Nic 1uM). PTX did not seem to affect Nic inhibition of K^+evoked Ca^{++}flux. Thus a PTX-sensitive G-protein seems to modulate Nic-MTX interaction in PC12 cells, without affecting Nic inhibition of K^+induced Ca^{++}rise.

457.4

FORSKOLIN AND 8-BROMO-cAMP ACT SIMILIARLY IN ENHANCING Ca^{++} CURRENTS IN BOVINE CHROMAFFIN CELLS. C.A Doupnik* and R.Y.K. Pun, Dept. of Physiology & Biophysics, University of Cincinnati, Cincinnati, OH 45267.

Recently, the modulation of ionic channels by forskolin has been suggested to be through a mechanism other than elevation of cytosolic cAMP. (Hoshi, T., et. al. Science 240:1652, 1988). To test whether forskolin affects voltage-dependent Ca^{++} currents in cultured bovine chromaffin cells via a cAMP-dependent mechanism, we compared the effects of forskolin with the cAMP analog 8-bromo-cAMP. Using the whole-cell voltage clamp technique, Ca^{++} currents were evoked by applying varying positive step pulses from a holding potential of -90 mV in the presence of 5 mM extracellular Ca^{++}. For each cell, a current-voltage (I-V) relation was determined before (control) and during local perfusion with forskolin (10 μM) or 8-bromo-cAMP (1 mM). Both compounds similiarly enhanced the peak currents; forskolin by 32 $\pm$ 5% (n=3) and 8-bromo-cAMP by 46 $\pm$ 17% (n=3). Neither compound however, significantly altered the voltage activation range. Half activation for the peak conductance occurred at -3 mV for control, -3 mV for forskolin, and -5 mV for 8-bromo-cAMP. In addition, using a single exponential sum of the least squares fit of the peak current decay, no significant differences were found between the calculated tau's of either compound. These preliminary results suggest that in chromaffin cells, forskolin acts via a cAMP-dependent mechanism and that Ca^{++} channel conductance is enhanced presumably via A-kinase phosphorylation of the Ca^{++} channel itself or an associated regulatory protein. Supported by NSF Grant DCB8812562.

457.5

DIFFERENTIAL EFFECTS OF ANTICONVULSANT AND CONVULSANT SUCCINIMIDES ON CALCIUM CURRENTS AND GABA RESPONSES OF THALAMIC NEURONS. D.A. Coulter, J.R. Huguenard, and D.A. Prince, Dept. Neurology, Stanford University Medical Center, Stanford, CA 94305.

We have previously reported that the petit mal anticonvulsants ethosuximide (ES) and dimethadione reduce the low-threshold, transient ("T") calcium current of thalamic neurons when applied in clinically relevant concentration ranges. This effect, coupled with the importance of the thalamic T current in thalamocortical oscillatory behavior (like the spike-wave discharges of petit mal), led us to hypothesize that the T current reducing action of these agents might be a mechanism mediating control of petit mal.

We analyzed the effects of the anticonvulsant succinimides ethosuximide (ES) and methylphenylsuccinimide (MPS) and the convulsant tetramethylsuccinimide (TMS) on calcium currents and GABA responses of acutely isolated rat thalamic neurons using voltage-clamp techniques. Thalamic neurons have at least two components of calcium current: a prominent T current, and a high-threshold, sustained ("L") current. MPS and ES reduced T current in a voltage-dependent manner, in clinically relevant concentrations. MPS was less potent than ES (IC_{50} of 1100 vs. 200 μM) but greater in efficacy (100% maximal reduction vs. 40% for ES). MPS was less specific than was ES, in that it also reduced the L current when applied in concentrations $\geq$ 500 μM. TMS had no effect on calcium currents except at very high concentrations, and did not occlude MPS effects when applied concurrently. TMS blocked GABA responses in a concentration-dependent manner, presumably accounting for the convulsant action of this drug. ES, in significantly higher concentrations, reduced GABA responses to a lesser degree, and occluded TMS block of GABA currents. This partial agonist action of ES on GABA responses may explain the ES anticonvulsant effect on chemically-induced seizures, but appears independent of the T current blocking actions of ES.

The finding that MPS, but not TMS, blocks T current provides additional support for the hypothesis that T current reduction is a cellular mechanism of anticonvulsant succinimides related to their effects in petit mal epilepsy. Supported by NIH Grants NS06477, NS12151, and NS07280.

457.6

COMPARISON BETWEEN THE ACTIONS OF ENDOTHELIN AND VASOPRESSIN IN PITHED RATS AFTER PRETREATMENT WITH BAY K 8644, NIFEDIPINE OR PERTUSSIS TOXIN. R. Tabrizchi* and C.R. Triggle. Faculty of Medicine, Memorial University of Newfoundland, St. John's, Newfoundland

The pressor actions of endothelin 3 (ET) and arginine vasopressin (AVP) were compared to one another in pithed rats in the presence of the calcium channel activator BAY K 8644 or the calcium channel antagonist nifedipine i.a. and also after pretreatment (-3 days) with pertussis toxin i.v. Administration of BAY K 8644 increased the diastolic pressure (DP) and nifedipine caused a decrease in DP. DP pressure recorded in pertussis toxin treated animals was significantly lower than saline treated animals. Both ET and AVP dose-dependently increased DP. The actions of ET but not AVP were potentiated in the presence of BAY K 8644, furthermore nifedipine significantly impaired responses induced by ET but not those produced by AVP. Treatment with pertussis toxin caused the dose-diastolic pressure response curve to ET to be displaced to the right whereas the dose-diastolic pressure response to AVP was not affected by treatment with pertussis toxin. These results suggest that extracellular calcium is partly responsible for the pressor response induced by ET, whereas the response to AVP appears to depend upon activation of an intracellular source of calcium. Furthermore it can be suggested that in vascular smooth muscle ET activates a pertussis toxin-sensitive G-Protein, whereas responses induced by AVP do not appear to be mediated through a G-Protein that is sensitive to pertussis toxin. (Supported by Canadian Heart Foundation)

457.7

EFFECTS OF MEMBRANE POLYUNSATURATED FATTY ACIDS ON $^{45}Ca^{++}$ UPTAKE INTO N1E-115 NEUROBLASTOMA CELLS. J. Gupta*, Z. Byczko*, and M.G. Murphy. Dept. of Physiology and Biophysics, Dalhousie University, Halifax, Nova Scotia, CANADA B3H 4H7

We have examined the effects of increasing the membrane content of w6 polyunsaturated fatty acids (PUFA) of neuroblastoma cells (clone N1E-115) on $^{45}Ca^{++}$ uptake under basal conditions, and in the presence of A23187 or bradykinin. Addition of $^{45}Ca^{++}$ (1μci/ml) to the culture dishes, following a 15 minute preincubation in medium containing 1.8 mM Ca^{++}, resulted in a biphasic uptake of $^{45}Ca^{++}$. Initially, there was an increase in Ca^{++} influx which reached a peak between 20 - 30 seconds. This was followed by a second phase of increase which was almost linear up to 5 minutes. Neither the magnitude nor the time course of uptake were altered in PUFA-enriched cells. A time dependent increase in $^{45}Ca^{++}$ uptake was seen in the presence of A23187. Exposure of cells to varying concentrations of bradykinin (10^{-5}M - 10^{-8}M) for different time periods did not have any effect on Ca^{++} uptake. PUFA-enriched cells did not exhibit altered $^{45}Ca^{++}$ uptake in the presence of A23187 or bradykinin.

(Supported by the Canadian MRC).

457.8

G PROTEIN-MEDIATED FMRF-AMIDERGIC MODULATION OF A CALCIUM CURRENT IN GIANT SYNAPSE. H.J. Man-Son-Hing and P.G. Haydon. Department of Zoology, Iowa State University, Ames, IA 50011.

The neuropeptide, Phe-Met-Arg-Phe-NH_2 (FMRFa) decreases the magnitude of a voltage-dependent calcium current and causes a presynaptic inhibition of transmitter release from cholinergic neuron B5 of *Helisoma* (Zoran et al., Neurosci. Abst. 1989; Haydon et al., Neurosci. Abst. 1989). In this study, we demonstrate that a G protein mediates FMRFa's action on the calcium current.

Various agents were included in the internal solution in the patch pipette during whole-cell recording from secretory somata. Four lines of evidence demonstrate G protein involvement in FMRFa's signal transduction pathway. 1) In the presence of either GTPγS (100μM; n=8) or GMP-P(NH)P (100μM; n= 5), FMRFa caused an irreversible decrease in the calcium current. 2) GDPβS (500μM; n=4) prevented FMRFa's action . 3) AlF_4 caused a rapid decline in the magnitude of the calcium current in the absence of FMRFa (n=8) and subsequent FMRFa application had no effect on the calcium current. 4) Addition of pertussis toxin prevented FMRFa's inhibition of the calcium current (n=6). These data demonstrate that a pertussis toxin-sensitive G protein is involved in mediating the inhibition of a calcium current by FMRF-amide.

Supported by a grant from the NIH, NS26650.

457.9

DEPHOSPHORYLATION OF VOLTAGE-DEPENDENT CALCIUM CHANNEL BY PURIFIED PROTEIN PHOSPHATASES. Y. Lai* and W.A. Catterall (SPON: B.M. Curtis). Department of Pharmacology, University of Washington, Seattle, WA 98195.

The Ca currents mediated by L-type voltage-dependent calcium channels (CaCh) have been shown to be modulated by protein phosphorylation in a variety of cells. The CaCh purified from skeletal muscle contains five specifically associated subunits. Two of the subunits, α_1 with M_r = 170,000 and β with M_r = 55,000, are phosphorylated by cAMP-dependent protein kinase. Stoichiometric phosphorylation of these subunits activates purified CaCh reconstituted in phospholipid vesicles (Nunoki et al., *Proc. Natl. Acad. Sci. USA*, in press). Because of the importance of regulation of channel function by protein phosphorylation, we have studied the dephosphorylation of Ca channel by purified protein phosphatases.

Purified CaCh was first phosphorylated by cAMP-dependent protein kinase and then incubated with purified protein phosphatase 1 (PP1), protein phosphatase 2A (PP2A), or calcineurin (CN). PP1 and PP2A dephosphorylated both α_1 and β subunits at rates comparable to those for the dephosphorylation of phosphorylase a, a physiological substrate of both protein phosphatases. Similar results were obtained when CaCh reconstituted in phospholipid vesicles or CaCh in skeletal muscle transverse tubules were used as substrates. Recent results suggest that CN dephosphorylates β subunits of CaCh more rapidly than α_1 subunits, but both rates are slower than those of PP1 and PP2A. We are currently examining the sites on α_1 and β subunits that are dephosphorylated by the different protein phosphatases.

457.10

SEROTONIN DECREASES WHOLE CELL CALCIUM CURRENT IN ACUTELY ISOLATED NEURONES FROM DORSAL RAPHE NUCLEUS OF ADULT RATS N.J. Penington* and J.S. Kelly. Dept. of Pharmacology, Univ. of Edinburgh, Med.Sch., Edinburgh EH8 9JZ, U.K.

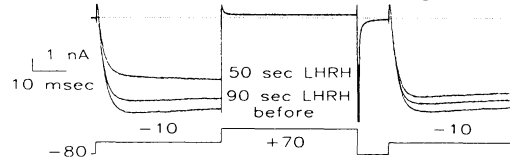
In peripheral and cultured central neurones and cell lines, voltage sensitive calcium currents ($I_{Ca}2+$) have been shown to be decreased or occasionally increased by several neurotransmitters and neuropeptides. Consequently, it is interesting to report that low concentrations of 5-HT and 8-OH-DPAT reduce $I_{Ca}2+$ in an isolated adult central neurone. 36 raphe neurones were voltage clamped and currents carried by Ca^{2+} (2mM) or Ba^{2+} (5mM) were isolated by blocking K^+ currents with external TEA and internal Cs^+. Cells were usually held at -100mV and stepped close to 0mV. 5-HT (10nM-200μM) was applied to 22 cells with a pipette (15μm tip). 10μM 5-HT reduced peak $I_{Ca}2+$ reversibly by up to 60% (n=8) with the largest effect occurring at the peak of the I/V relationship. Application of control solution had no comparable effect and 8-OH-DPAT (10-1000nM) the selective 5-HT_{1A} agonist had the same action as 5-HT (n=4). At the peak of the I/V relationship 5-HT caused in most cells a selective inhibition of a decaying component of $I_{Ca}2+$ (inactivated at a h.p. of -50mV (N-like)). In others, 5-HT caused a marked slowing of $I_{Ca}2+$ activation. This implies that 5-HT may alter the proportion of channels inactivated by current or voltage and hence the number capable of opening near the peak of the I/V relationship. Supported by the Wellcome Trust.

457.11

LHRH AND GTP-γ-S MODIFY CALCIUM CURRENTS OF ISOLATED FROG SYMPATHETIC NEURONS. Keith S. Elmslie, Wei Zhou*, and Stephen W. Jones. Dept. Physiology & Biophysics, Case Western Reserve U., Cleveland, OH 44106.

Chicken II luteinizing-hormone releasing hormone (LHRH) reduces whole-cell calcium current in frog sympathetic neurons, but only partially (~50%), and the remaining current activates more slowly. Similar results have often been interpreted as selective inhibition of a rapidly inactivating calcium channel. We suggest instead that the kinetics of a single type of current are modified, since (1) the kinetics become *more* complex in LHRH, not *less*, as activation is fit by the sum of two exponential processes rather than one, and (2) strong depolarizing prepulses largely reverse the effects (see Figure). One possibility is that, at negative membrane potentials, LHRH shifts a fraction of the channels into a state from which opening is delayed. Similar kinetics can be achieved by internal dialysis with GTP-γ-S.

Figure: Effect of 100 nM LHRH on whole-cell current (2mM Ba^{2+}). Note the slow kinetics and rapidly desensitizing response.



457.12

CONCENTRATION-DEPENDENT EFFECTS OF METHIONINE-ENKEPHALIN ON $[Ca^{2+}]_i$ OF ENTERIC NEURONS; INVESTIGATIONS WITH FURA-2. K.Hirai* and Y.Katayama, Dept. Autonom. Physiol., Med. Res. Inst., Tokyo Med. and Dent. Univ., Tokyo, 101 JAPAN.

Fura-2 measurement of $[Ca^{2+}]_i$ was made simultaneously with intracellular recordings from single neurons. Submucous plexus preparations were made from the isolated caecum of the adult guinea-pig sacrificed by heavy stunning and bleeding from the neck.

Action potentials elicited a transient increase in the $[Ca^{2+}]_i$ ($\Delta[Ca^{2+}]_{AP}$). Low concentration of met-enkephalin (ME, 1-30 nM) induced a small membrane hyperpolarization, a $[Ca^{2+}]_i$-increase and a $\Delta[Ca^{2+}]_{AP}$-augmentation. ME at 100 - 300 nM hyperpolarized the membrane, decreased the $[Ca^{2+}]_i$ and attenuated the $\Delta[Ca^{2+}]_{AP}$. When the hyperpolarization was nullified, the $[Ca^{2+}]_i$ increased and the attenuation of the $\Delta[Ca^{2+}]_{AP}$ was abolished. ME more than 1 μM irreversibly depressed the $\Delta[Ca^{2+}]_{AP}$. The concentration-dependent effects of ME on the $\Delta[Ca^{2+}]_{AP}$ may explain the findings that ME facilitates the sympathetic ganglionic transmission at low doses but inhibits it at high doses.

457.13

ENHANCEMENT OF RAT BRAIN CALCIUM CHANNEL ACTIVITY IN XENOPUS OOCYTES BY A PROTEIN KINASE C ACTIVATOR. Bradford P. Schmidt* and John P. Leonard(SPON: C.J. Siok) Department of Biological Sciences. University of Illinois at Chicago, Chicago, Illinois 60680

Xenopus oocytes were used as expression vectors for calcium channels encoded by exogenous rat brain RNA. Endogenous Ca channel activity in oocytes ranges from undetectable up to 50 nA while brain RNA-injected oocytes contain Ca channels exhibiting different properties with currents ranging from 200 to 1000 nA. The effect of 100 nM phorbol ester dibutyrate (PDBu), a C-kinase activator, on both endogenous and RNA-induced Ca channels was investigated. Oocytes were injected with RNA purified from the brains of two-week old rats. Currents through voltage-sensitive Ca channels were observed using two-electrode voltage-clamp. Barium was used in place of Ca since Ba often permeates Ca channels better than Ca and because Ba is a poor activator of an interfering Ca-dependent Cl current. To rule out any interference of the Cl current and possible effects on Na-dependent exchangers, experiments were done both in the presence and absence of Cl_o and/or Na_o. Controls consisted of application of the inactive stereoisomer 4-α-phorbol. Ba currents were enhanced to 142% and 137% of control currents for brain RNA-injected and non-injected oocytes, respectively. No change in the voltage dependence of activation or voltage of maximal activation was observed. (Supported by NIH NS26432)

457.14

PROSTAGLANDIN E_2 INHIBITS A CALCIUM CURRENT IN ADULT RAT SUPERIOR CERIVCAL GANGLION (SCG) NEURONS. S.R. Ikeda. Department of Pharmacology and Toxicology, Medical College of Georgia, Augusta, GA 30912.

Prostaglandins (PG) have been demonstrated to be important modulators of autonomic neurotransmission (reviewed by Brody, J. and Kadowitz, P.J., *Fed. Proc.* **33**:48-60, 1974). Prostaglandins of the E series: 1) are released during autonomic nerve stimulation, 2) decrease norepinephrine release, and 3) modify sympathetic neuron electrical excitability. In this study, the effect of PGE_2 on the voltage-activated Ca^{2+} current of rat SCG neurons was investigated.

SCG neurons were acutely isolated from adult rats by enzymatic dispersion. Ca^{2+} currents were recorded using the whole-cell variant of the patch-clamp technique in solutions designed to isolated Ca^{2+} currents. Ca^{2+} currents were evoked from a holding potential of -80 mV by depolarizing command steps. Under these conditions, introduction of 0.1-1.0 μM PGE_2 produced a rapid and reversible inhibition of the Ca^{2+} current. Mean inhibition of current was 41±3 and 54±2%, respectively for 0.1 and 1.0 μM PGE_2 (measured isochronally 10 msec after a command to +10 mV). Maximal block was produced at the peak of the current/voltage curve (+10 to +15 mV). In addition, PGE_2 produced an apparent slowing of the rising phase of the current at potentials positive to +10 mV. If similar events occur at adrenergic nerve terminals, these results may explain the presynaptic inhibitory action of PGE_2. (Supported in part by a grant from the Pharmaceutical Manufactures Association Foundation)

457.15

Adenine and Guanine Nucleotides Reduce Voltage-Dependent Calcium Current in Isolated Rat Purkinje Neurons. P. Hockberger[1], L. Yousif,*[1] and S. Christakos.*[2] [1]Dept. of Physiology, Northwestern University Medical School , Chicago, IL 60611 and [2]Dept. of Biochemistry , New Jersey Medical School, Newark, NJ 07103.

Calcium currents were analyzed in cerebellar Purkinje cell bodies (PCs) acutely isolated from rats between postnatal days 7-13 following papain digestion (cf. Regan, L. Soc. Neurosci. Abstr. #31.7, 1987). Isolated PCs were identified using immunocytochemical staining for the PC-specific antigens PEP-19 and calbindin (1:2000 and 1:20,000 antibody dilution, respectively). Whole-cell recordings were performed as described elsewhere (Hockberger, P., Toselli, M., Swandulla, D. and Lux, H.D., Nature 338:340, 1989). High threshold (L-type) calcium currents were recorded from all cells examined at this stage of development (n=64). Low threshold (T-type) calcium currents were present in 28% of the cells (n=18). Extracellular application of 10^{-3}M 8-bromo-cGMP via a micropipet reduced the L-type current by 20-60% (n=27). Applications lasting several seconds resulted in reductions that recovered completely within 10 -20 sec, and this could be repeated several times without affecting the speed of recovery. Similar effects were induced by 10^{-3}M 8-bromo-cAMP, while weaker responses (10-20% decreases) were obtained with cGMP, 5'GMP, 5'AMP (10^{-3}M ea.), or IBMX (10^{-4}M). The adenosine analogue 2-chloroadenosine (10^{-4}M) was ineffective. Including 8-bromo-cGMP in the internal saline, or delivering it intracellularly through a second patch pipet, was also ineffective. These results suggest that PCs may have external nucleotide receptors that are coupled to L-type calcium channels.

This research is supported by NIH grants nos. NS-26915 & RR-05370 (PH), and NS-20270 (SC).

457.16

COUPLING OF NEUROTRANSMITTER RECEPTORS TO Ca^{2+} CHANNELS IN DORSAL ROOT GANGLION NEURONS USING PURIFIED RECOMBINANT G-PROTEINS. Maurine Linder†*, Alfred G. Gilman†*, Douglas A. Ewald and Richard J. Miller, Department of Pharmacology, University of Texas, Dallas† and Department of Pharmacological and Physiological Sciences, University of Chicago, Chicago, IL 60637.

We measured the effects of the neuropeptides bradykinin (BK) and neuropeptide Y (NPY) on Ca^{2+} currents (I_{Ca}) in rat dorsal root ganglion (DRG) neurons in vitro using the whole cell voltage clamp technique. In control neurons both of these peptides inhibited the I_{Ca} by about 60%. When cells were treated with pertussis toxin the inhibitory effects of both NPY and BK were completely abolished. We examined the role of G-proteins in the coupling of receptors to Ca^{2+} channels in these cells by using purified recombinant (r) G-protein α-subunits expressed in E. coli. We attempted to reconstitute receptor mediated inhibition of the I_{Ca} in pertussis toxin treated neurons using $r\alpha_o$, $r\alpha_{i1}$, $r\alpha_{i2}$ or $r\alpha_{i3}$ introduced into cells with a patch pipette. When any of these proteins were perfused into neurons (100nM), the BK induced inhibition of the I_{Ca} returned in a time dependent fashion. In contrast there was a considerable selectivity in the ability of the proteins to reconstitute inhibition produced by NPY. $r\alpha_o$ was the most potent followed by $r\alpha_{i1}$ and $r\alpha_{i3}$. However $r\alpha_{i2}$ was unable to couple NPY receptors to DRG Ca^{2+} channels. The effects of $r\alpha_o$ were manifest between 10 and 100 nM with an EC_{50} of about 30 nM. High concentrations of $r\alpha_{i1}$, $r\alpha_{i2}$, $r\alpha_{i3}$ or $r\alpha_o$ did not inhibit forskolin-stimulated adenylate cyclase purified from bovine brain suggesting that hormonal inhibition of adenylate cyclase is not directly mediated by an α_i/cyclase interaction. Furthermore the coupling effects observed with recombinant proteins confirm ($r\alpha_o$, $r\alpha_{i1}$, $r\alpha_{i2}$) and extend ($r\alpha_{i3}$) our previous studies on the selectivity of BK and NPY receptor coupling to Ca^{2+} channels (Neuron, 2, 1185, 1989).

457.17

MODULATION OF SYNAPTOSOMAL CALCIUM FLUX BY ARGININE VASOPRESSIN. M.L. Koenig, M.A. Oleshansky, and G.S. Dhillon, and M.A. Jackson*. Dept. Medical Neurosciences, Walter Reed Army Institute of Research, Washington DC 20307.

The neurohypophysial hormone arginine vasopressin (AVP) has been shown to have Ca^{2+}-mobilizing properties and to affect certain CNS processes including those involved in learning and memory. We have investigated the possibility that AVP can modulate Ca^{2+} influx in synaptosomes prepared from rat cortex and striatum.

$^{45}Ca^{2+}$ influx was measured in crude (P2) synaptosomal fractions which had been preincubated with AVP at 30°C for 10 min. AVP (1 μM) increased the net depolarization-dependent influx of $^{45}Ca^{2+}$ into cortical synaptosomes by 20% (1.87 + 0.09 vs 1.56 + 0.12 nmol/mg protein) when influx was terminated after a 1 sec exposure to radiolabelled Ca^{2+}. The peptide had no effect on net $^{45}Ca^{2+}$ influx measured at 15 sec. $^{45}Ca^{2+}$ influx in striatal synaptosomes was not affected by AVP at either 1 sec or 15 sec.

Separate experiments in our laboratory have demonstrated that AVP does not affect adenylate cyclase activity in cortical synaptosomes. AVP may be directly influencing the activity of the fast phase synaptosomal Ca^{2+} channel.

457.18

THREE COMPONENTS OF Ca-CHANNEL CURRENT AND THEIR MODULATION BY A GTP-BINDING PROTEIN. H. Kasai. Max-Planck-Institut für biophysikalische Chemie, D3400 Göttingen, FRG.

Ca-channel currents of mouse neuroblastoma NG108-15 cells were recorded with the whole-cell patch-clamp method using 10 mM Ba as a charge carrier. These Ca-channel currents could be separated into three components according to their sensitivity to dihydropyridines (DHPs) and w-conotoxin GVIA (wCTX). One component, $I_{\omega CTX}$, was irreversibly blocked by wCTX, resistant to DHPs, and activated at potentials more positive to -20 mV. A second component, I_{DHP}, was sensitive to DHPs, resistant to wCTX, and activated at -30 mV. Both $I_{\omega CTX}$ and I_{DHP} were sustained during depolarization. In contrast, a third component, I_{LVA}, was resistant to both wCTX and DHPs and was activated at -50 mV. I_{LVA} was inactivated in a voltage-dependent manner, being completely eliminated at a holding potential of -40 mV. The δ-opiate agonist, DPDP-Enkephalin, could reduce all three components of Ca-channel current. However, its effects could be specific to certain components of currents in certain cells and culture conditions. Reduction of $I_{\omega CTX}$ and I_{LVA} was voltage- and time-dependent and relieved by depolarization, while the reduction of I_{DHP} was voltage- and time-independent. All these effects were blocked by pertussis toxin. These results suggest that the three Ca-channels of this cell exhibit sensitivities to DHPs and ωCTX which differ from the expectations of the T, L and N-classification. These three Ca-channels can be modulated in different ways by a GTP-binding protein.

457.19

LOCALIZATION OF CALCIUM INFLUX THROUGH PHORBOL ESTER-INDUCED CALCIUM CHANNELS. L.A. Fink*, L.K. Kaczmarek, and J.A. Connor. Department of Pharmacology, Yale University School of Medicine, New Haven, CT 06510; Roche Institute of Molecular Biology, Nutley, NJ 07110

Activators of protein kinase C have been shown to enhance the calcium current in the bag cell neurons of Aplysia by unmasking a 24 pS voltage-dependent calcium channel in the plasma membrane. This species of channel is not observed in control cells in which the kinase has not been stimulated, although control cells possess calcium channels of lower unitary conductance (~12 pS). We have now used fluorescent imaging of the calcium-indicator fura-2 to compare the localization of calcium influx in control cells and in cells exposed to the protein kinase C activator, TPA (20 nM). Single bag cell neurons with extensive neuritic outgrowth after one day in culture were loaded with fura-2 through a microelectrode, and fluoresecent ratio images were generated with a cooled CCD-based imaging system. In control cells, a train of action potentials generated large increases in the calcium signal from the neurites, with little or no increase in calcium at the soma. In contrast, after exposure of the cells to TPA for 10-15 min, much larger elevations of calcium at the soma were observed in response to action potentials. The depolarization-induced elevation of calcium in the neurites was also enhanced by TPA. Our data suggest that the large conductance calcium channels induced by activators of protein kinase C are located both on the soma and the neurites of isolated bag cell neurons, whereas, calcium influx through the lower conductace channels in both control and activated cells occurs primarily in the neurites.

457.20

CHARACTERIZATION OF AN α-ADRENERGIC RECEPTOR MEDIATED DECREASE OF THE CALCIUM CURRENT OF ACUTELY ISOLATED ADULT RAT SUPERIOR CERVICAL GANGLION NEURONS. Geoffrey G. Schofield. Department of Physiology, Tulane University Medical School, New Orleans, LA 70112.

At sympathetic neuroeffector junctions norepinephrine can decrease transmitter release by the activation of α_2-adrenoceptors located on synaptic varicosities. Norepinephrine can also decrease the Ca^{2+} current recorded from neuronal somata via an α-adrenoceptor. However, the receptor subtype involved in the norepinephrine induced decrease of the Ca^{2+} current has not been fully characterized. Ca^{2+} currents were recorded from single neurons, acutely isolated from adult rat superior cervical ganglia (SCG) using the whole-cell patch-clamp technique. The Ca^{2+} current induced by step depolarizations to +10 mV from a holding potential of -80 mV was decreased by 1 μM norepinephrine by 50.1 ± 4.5%. In the presence of the α_2-adrenoceptor antagonist idazoxan, 1 μM norepinephrine reduced the Ca^{2+} current amplitude by 8.9 ± 2.2%. On the other hand, the norepinephrine-induced decrease of the Ca^{2+} current was not attenuated by the α_1-adrenoceptor antagonist prazosin (1 μM). Idazoxan and prazosin themselves did not decrease the Ca^{2+} current. The α_2-adrenoceptor agonist clonidine (1 μM) also induced a decrease of the Ca^{2+} current of 34.0 ± 3.3%, whereas the α_1-adrenoceptor agonist phenylephrine (1 μM) decreased the Ca^{2+} current by only 13.0 ± 5.9%. These results suggest that norepinephrine decreases the Ca^{2+} current of adult rat SCG neurons via an α_2-adrenoceptor. Supported by the Pharmaceutical Manufacturers Association Foundation.

457.21

WITHDRAWN

LIGAND-GATED ION CHANNELS II

458.1

THE BENZODIAZEPINE DIAZEPAM AND THE β-CARBOLINE DMCM MODULATE $GABA_A$ RECEPTOR SINGLE CHANNEL CURRENTS BY OPPOSITE MECHANISMS. C.J. Rogers, R.E. Twyman and R.L. Macdonald. Dept. of Neurology, Univ. of Michigan, Ann Arbor, MI 48104.

Diazepam (DZ) and DMCM bind to the benzodiazepine binding site of the $GABA_A$ receptor channel and increase and decrease GABA receptor current, respectively. The effects of DZ and DMCM on single channel $GABA_A$ receptor currents were studied to determine their mechanisms of action.

Outside-out patches from mouse spinal cord neurons grown in cell culture were held at -75 mV in symmetric chloride solutions. Data were digitized at 20 kHz with 2 kHz Bessel filtering. Data from different patches were pooled for descriptive and kinetic analyses. Only the main conductance state (~27 pS) was analyzed. DZ or DMCM (20-100 nM) was included in the bathing medium. GABA (2μM) alone or GABA plus DZ or DMCM was applied from pressure ejection pipettes to the patch.

DZ and DMCM increased and decreased, respectively, the frequency of GABA receptor single channel currents in a concentration dependent manner without altering mean open time. Open durations of $GABA_A$ receptor currents were placed into an open time frequency histogram best fit by three exponential functions similar for GABA, GABA+DZ and GABA+DMCM and with similar relative frequencies of occurrence. $GABA_A$ receptor currents occurred in bursts of openings (separated by closures greater than 5 msec) and bursts occurred in clusters (separated by closures greater than 50 to 150 msec). Burst and cluster frequencies were increased and decreased by DZ and DMCM, respectively. These data suggest that the major effect of DZ is to increase the affinity of GABA for the $GABA_A$ receptor, an effect opposite to that of DMCM which predominantly decreases the affinity.

458.2

CYCLIC AMP DEPENDENT PROTEIN KINASE DECREASES $GABA_A$ RECEPTOR CURRENT IN MOUSE SPINAL NEURONS. N.M. Porter, R.E. Twyman, M.D. Uhler*, and R.L. Macdonald. Dept. of Neurology and the Mental Health Research Institute*, Univ. of Michigan, Ann Arbor, MI 48104

The predicted structure of the neuronal bovine $GABA_A$ receptor places a consensus phosphorylation site for cAMP dependent protein kinase (PKA) on an intracellular domain of the receptor. To determine the role of PKA-mediated phosphorylation on $GABA_A$ receptor function, we measured GABA receptor chloride currents in the presence of PKA in mouse spinal neurons using the whole cell and patch clamp techniques. The bath medium contained (mM): 142 NaCl, 8.1 KCl, 1 $CaCl_2$, 6 $MgCl_2$, 10 glucose, and 10 HEPES; the recording pipette contained: 153 KCl, 1 $MgCl_2$, 2 Mg-ATP, 5 EGTA, and 10 HEPES. In whole cell recordings, application of GABA (5 μM) to the soma reproducibly evoked an inward chloride current and an increase in membrane conductance. Inclusion of the purified catalytic subunit of PKA (50 μg/ml) in the pipette solution reduced the peak conductance evoked by GABA by 59% (n = 35). The GABA receptor current was not reduced, however, in cells in which heat-inactivated catalytic subunit was substituted for the active subunit in the pipette solution (n = 8). Application of GABA to excised, outside-out patches evoked bursting inward chloride currents in control patches (n = 10). In PKA patches very few openings were recorded and the total GABA receptor current was reduced by 97% (n = 7). PKA decreased GABA receptor current primarily by reducing the frequency of channel opening.

These results suggest that phosphorylation of the $GABA_A$ receptor, or of a protein associated with the channel, regulates chloride channel opening. *Supported by NIH NS08216 to NMP and DA04122 to RLM.*

458.3

SINGLE CHANNEL INTRABURST KINETIC MODELLING OF THE $GABA_A$ RECEPTOR MAIN CONDUCTANCE STATE R.E. Twyman, C.J. Rogers and R.L. Macdonald. Dept of Neurology, Univ of Michigan, Ann Arbor, MI.

To determine gating kinetics of the $GABA_A$ receptor channel, intraburst properties of the main conductance state (27 pS) were investigated with the outside-out patch clamp recording technique. Patches obtained from mouse spinal cord neurons grown in cell culture were held at -75 mV in symmetric chloride solutions. Data were digitized at 8 kHz with a 1 kHz Bessel filter interposed. Frequency distributions of openings per burst, all intraburst openings, individual openings, successive openings and total open time of bursts containing 1 to 5 openings were analyzed by curve fitting to sums of geometric, exponential or gamma functions. Intraburst closed times were analyzed similarly.

The analysis confirmed the presence of 3 concentration independent open states (dwell times of 1.0, 2.9 and 8.1 msec) and 2 intraburst closed states (0.2 and 2.0 msec). The results suggested that the GABA receptor main conductance state opens into at least one brief open state from a singly liganded closed state and two longer duration open states from doubly liganded closed states. Channel openings were grouped into bursts with three different mean durations. Bursts were formed primarily by repeated openings of individual open states. The shortest open state opened on average 1.1 times, while the medium and longest duration open states opened on average 2.0 and 3.9 times, respectively. The brief intraburst closures may represent open channel blocked states or channel closed states which are independent of ligand associated gating. Each open state bursts, therefore, due to channel blocking or intrinsic channel closure rather than due to ligand associated channel gating. A kinetic model consistent with these observations will be presented.

458.4

DELTAMETHRIN ALTERS PENTOBARBITAL-INDUCED CHLORIDE CURRENTS IN CULTURED HIPPOCAMPAL NEURONS. J.M.Frey and T. Narahashi. Dept. of Pharmacology, Northwestern Univ. Med. Sch., Chicago, IL 60611.

Alpha-cyano pyrethroid insecticides have been shown to interact with the GABA/Cl^- receptor-ionophore in a complex manner. We have found that the acute application ($\leq$ 2 min) of the cyano pyrethroids fenvalerate and deltamethrin [DM] at concentrations up to 50uM can enhance (not suppress as one might expect) GABA-activated Cl^- currents in cultured hippocampal [HC] neurons (Frey et al., *Toxicologist*, 9:149, 1989). In an effort to understand this phenomenon, we tested the effect of DM (1-100uM) on pentobarbital [PB]-induced Cl^- currents in HC neurons recorded in the whole cell mode. Application of PB (100-500uM) by puffer for 20-30 sec elicited inward currents (0.2-1.2nA) which reversed at the Cl^- equilibrium potential and were blocked by both picrotoxinin and bicuculline. DM dramatically enhanced PB-induced peak currents when co-applied with PB (>60% at 10uM). This increase in peak current was accompanied by an increase in the rate of decay of the falling phase of the response. Currents were also enhanced when DM was pre-applied for 2-3 min, followed by co-application of PB/DM; however, this enhancement was oftentimes much less than co-application alone. In such cases, pre-application of DM for 2-3 mins often resulted in a reduction (<25%) in PB-induced current, suggesting competing mechanisms. These results implicate the PB binding site as a site of action of the cyano pyrethroid insecticides. Supported by NIH grant NS14143.

458.5

LINDANE AND CYCLODIENE INSECTICIDES BLOCK GABA-ACTIVATED CHLORIDE CURRENT IN CULTURED RAT HIPPOCAMPAL NEURONS. T. Narahashi and J.M. Frey. Dept. of Pharmacology, Northwestern Univ. Med. Sch., Chicago, IL 60611.

Lindane blocks GABA-activated Cl^- currents in cultured neurons (Ogata et al., *FASEB J.*, 2:2896, 1988; Frey et al., *Toxicol*. 9:149, 1989). We have compared the effects of lindane and the cyclodienes in cultured rat hippocampal neurons using the whole cell patch clamp technique. GABA (15 μM), delivered by puffer for 20-30 sec, produced rapid inward currents with distinct early transient (T) and late sustained (S) components (see Dichter and Frey, this meeting). Both T and S components were reduced reversibly (50 and 30%, respectively, 10 μM) by either coapplication or a single 2 min preapplication of lindane. The cyclodienes dieldrin (HEOD), endrin, heptachlor-epoxide (HE) and isobenzan (1-50 μM) also reduced GABA/Cl^- currents with a relative potency of endrin = isobenzan > HEOD = HE. However, the onset of action of the cyclodienes was much slower than lindane. Continuous application of HE or HEOD at 10 μM blocked 50% of the T component in ~6 min, and the current continued to decrease to <40% over 9-12 min. The S component was reduced by <25% within 2 min but remained unchanged with subsequent application. The effects on both components were irreversible. Thus, both lindane and the cyclodienes blocked GABA/Cl^- currents although their time course of action were markedly different. The results are compatible with the convulsant action of these insecticides. Supported by NIH grant NS14143.

458.6

ALLOSTERIC INTERACTIONS BETWEEN THE GABA, CHLORIDE AND BICYCLOPHOSPHATE BINDING SITES IN RAT BRAIN. R.G.Thompson, D.E. Menking* and J.J. Valdes. Biotechnology Div., Chem., Res. Devel. & Engr. Ctr., Aberdeen Proving Ground, MD 21010-5423.

This study investigated the degree to which GABA-A receptor agonists, chloride ions and bicyclophosphate compounds can influence the in vitro binding of radioligands to the multimeric GABA/anion/picrotoxin/benzodiazepine receptor complex isolated from rat brain.

Chloride ions, while stimulating membrane binding of ^{35}S-TBPS, inhibited ^{3}H-MUS binding to the GABA receptor by a maximum of 33% at 500 mM. Chloride also fully protected the ^{35}S-TBPS binding sites from heat inactivation (60^{o}C,30 min) but provided no protection for the GABA receptor. Cold TBPS also showed partial inhibition of ^{3}H-MUS binding to the GABA receptor and partially antagonized the effects of Cl^-. In addition, TBPS provided a small (10-30%) protective effect against thermal inactivation of the GABA receptor. Incubation of the membranes with cold MUS completely inhited ^{35}S-TBPS binding and also protected 67% of the binding sites from heat inactivation. Neither GABA receptor agonist nor the bicyclophosphates showed an ability to protect their own respective binding sites from heat inactivation.

In contrast to the interactions observed between Cl^- and bicyclophosphates on the receptor-gated ion channel, the results of studies on membrane preparations containing a voltage-gated Cl^- channel will also be presented.

458.7

SEROTONIN DIRECTLY EXCITES SPINAL MOTONEURONS. A.J. Berger and T. Takahashi* Dept. of Physiol., Faculty of Medicine., Kyoto Univ., Kyoto, Japan.

Serotonin (5-HT) increases excitability of spinal motoneurons (MNs) (Barasi, et al., Br. J. Pharmacol. 52: 339, 1974), but the mechanism for this effect is unknown. We studied this by applying whole-cell recording to neonatal rat MNs visually identified in thin spinal cord slices. In current clamp mode, bath-applied 5-HT (10 μM) in nominally Ca-free Mg (5 mM) Krebs solution depolarized MNs beyond firing threshold. In voltage clamp, at approx. -90 mV in Ca-free Krebs containing TTX, 5-HT generated an inward current (I_{5-HT}) associated with a conductance increase. External application of Cs (10 mM) but not Ba (2 mM) virtually abolished the I_{5-HT}. At higher external K concentrations (K_o), peak amplitude of the I_{5-HT} was larger. The I_{5-HT} current-voltage relation showed inward rectification. In 20 mM K_o, the I_{5-HT} reversal potential was -29 mV (n = 7 MNs). These I_{5-HT} characteristics are similar to those of the inward rectifying current seen in these MNs during membrane hyperpolarization. We conclude that 5-HT directly excites spinal MNs and this is probably mediated by the inward rectifying current present in MNs.

(Supported by Grant No. 63641515 from the Ministry of Education, Science and Culture of Japan to TT, and a USPHS Fogarty Senior Fellowship to AJB)

458.8

D_2 DOPAMINE RECEPTOR ACTIVATED K^+ CHANNELS IN SUBPOPULATIONS OF FRESHLY DISSOCIATED RAT CORPUS STRIATUM NEURONS. Jonathan E. Freedman and Forrest F. Weight. Sect. Electrophysiol., Natl. Inst. on Alcohol Abuse & Alcoholism, Rockville, MD 20852.

The corpus striatum (caudate and putamen) is a heterogeneous cell population rich in postsynaptic dopamine receptors. We have previously described an 85 pS K^+ channel activated by D_2 dopamine receptors in striatal neurons freshly dissociated from young adult rats (*Proc. Natl. Acad. Sci.*, **85**: 3618). We have now compared cells obtained by various modifications in our isolation procedure in order to (i) optimize dissociation conditions and (ii) assess whether this response is associated with a particular subtype of striatal neuron.

Among the modifications tested were cutting thin (100-250 μm) tissue slices on a vibrotome, treating the slices with less trypsin than in our original study (0.1-0.5 concentration and 0.5 incubation time), and substituting glucose or sucrose for NaCl in the dissociation medium in an attempt to decrease any ischemic or excitotoxic damage. Combinations of these methods increased cell yield, with greater diversity of cell size and shape, and better preservation of dendritic structure.

Cell-attached patch-clamp recordings indicated that not all dissociated cells displayed the D_2-activated channel; the proportion of responsive cells varied with the dissociation procedure. This raises the possibility that the response may be localized to a subpopulation of striatal neurons which survives various isolation procedures differentially. We are presently assessing whether D_2 responses are associated with a morphologically identifiable cell subtype, and whether other cells might have responses mediated by D_1 receptors.

458.9

ATP-ACTIVATED CHANNELS FROM PARASYMPATHETIC CARDIAC NEURONS. L.A. Fieber and D.J. Adams*, Dept. of Pharmacology, Univ. of Miami Sch. of Med., Miami, FL 33101.

Extracellular ATP activates a cation-selective channel in cardiac neurons of rat parasympathetic ganglia. ATP-activated whole cell currents exhibit inward rectification and a reversal potential near 0 mV. The characteristics of ATP-activated currents and ACh (nicotinic)-activated currents are similar, though pharmacologically distinct. The ATP-induced current is reduced during bath application of the non-hydrolyzable ATP analog α-β-methylene ATP and blocked by Reactive Blue (Kd 1 μM). Single ATP-activated channels were recorded in cell-attached and excised patches from cultured cardiac neurons. The patch pipette contained (mM) 140 CsCl, 10 HEPES-CsOH, pH 7.0 with 5 μM ATP. ATP-activated channels obtained from excised patches in a Ca-free bath solution had linear current-voltage relationships and a conductance of about 30 pS. Similar results were obtained from cell-attached patches. The reduction in the probability of opening (P_o) observed at depolarized membrane potentials may underlie the rectification of the whole-cell ATP-evoked current. The sustained activity of ATP receptor-channels in excised membrane patches suggests that ATP receptors are directly coupled to ion channels and do not require the continued presence of a cytosolic second messenger.

458.10

A PROTON-ACTIVATED SUSTAINED INWARD CURRENT IN RAT DORSAL ROOT GANGLION NEURONS. *S. Bevan* and J.C. Yeats** (SPON: Brain Research Association), Sandoz Institute for Medical Research, 5 Gower Place, London WC1E 6BN, UK.

A reduction in the external pH of the medium bathing rat and chick DRG neurons to <pH 7 elicits a transient inward current, which relaxes in 1-2s (Krishtal & Pidoplichko, 1980; Konnerth et al., 1987). We have found that lowering the pH to <6.4 also activates a sustained inward current in a sub-population of DRG neurons.

3-7 day old cultures of neonatal rat DRG neurons responded to acidification of the external medium to <pH 6.4 with an efflux of ^{14}C-guanidinium and ^{86}Rb ions. This efflux was abolished when cultures were pretreated for 24 hours with 2-10μM capsaicin to kill the capsaicin-sensitive cells. Freshly isolated adult rat DRG neurons were also examined by whole cell voltage clamp methods. In addition to the transient current, acid solutions (<pH 6.2) evoked a more sustained inward current in 30-50% of neurons studied at a holding potential of -80mV, with a standard NaCl-based external solution and either a CsCl- or KCl-based internal solution. Under these ionic conditions the reversal potential for this current was close to 0mV. Experiments were also made with NaCl solutions on both sides of the membrane: reduction of the external NaCl solution to 50mM from 130mM (with isosmotic replacement with sucrose) shifted the reversal potential for the sustained pH response by about -23mV which is close to the value expected for a cation selective conductance. We conclude that protons activate a relatively non-specific cation conductance in a sub-population of DRG neurons that are restricted largely, if not exclusively, to the capsaicin sensitive population.

458.11

VOLTAGE- AND RECEPTOR-ACTIVATED CURRENTS IN AR42J CELLS. K. Kusano and H. Gainer. LNC, NIH, NINDS, Bethesda, MD 20892.

Membrane currents were studied in AR42J cells, a rat pancreatic acinar cell line. A whole-cell configuration of the patch-electrode voltage clamp technique was used. The AR42J cells exhibited the following membrane voltage-activated ionic currents: $I_{K(Ca)}$, I_{Na}, $I_{Ca(t)}$ and $I_{Cl(Ca)}$. These currents were identified by their ionic dependencies, pharmacological properties and kinetic parameters. Spontaneous action potentials were seen in a small number of cells under current clamp condition. Receptor-activated membrane currents were studied by applying the following ligands: sulfated CCK-8(10^{-6}M), bombesin (10^{-6}M), substance-P ($5x10^{-8}$M), secretin (10^{-5}-10^{-6}M), vasopressin (10^{-6}M), and acetylcholine (10^{-4}M). Most of these ligands are known to be pancreatic secretagogues. All of these ligand-activated receptor currents were inward at the membrane holding potential of -70 mV, and were accompanied by a membrane conductance increase. Receptor-activated currents typically were inactivated within 30 sec in the presence of the ligands. The reversal potential of the I_{CCK} obtained from 8 cells was about 0 mV or slightly positive, but in 2 cells it was between -20 and -25 mV. Such reversal potentials were also observed for $I_{bombesin}$ and for I_{ACh}. It appeared that these three secretagogues activated similar ion channels. However, there was some variation between the cells with respect to their ionic selectivity and permeability. The above receptor-activated currents could not be recorded in "Ca-free" medium or with a pipette containing EGTA, but could be recorded in the presence of 500 μM Cd^{2+} in control saline which did block the calcium current. This suggests that receptor activation produces a rise of Ca^{2+} by the release of Ca^{2+} from intracellular stores. Thus AR42J cells possess many electrophysiological properties which are similar to primary rat pancreatic acinar cells, but have several currents (I_{Ca}, I_{Na}) not yet reported for the primary cells.

458.12

ISOLATION OF THE 58K SUBUNIT OF THE GLYCINE RECEPTOR OF RAT SPINAL CORD. G. Grenningloh*, P. Prior*, I. Pribilla* and H. Betz. (SPON: C.S. Goodman). ZMBH, Universität Heidelberg, Im Neuenheimer Feld 282, D-6900 Heidelberg, Fed. Rep. of Germany.

The inhibitory glycine receptor is composed of three polypeptides of 48K, 58K and 93K. The 48K and 58K subunits are transmembrane proteins combined in a pentameric complex which forms the glycine-gated chloride channel. The ^{3}H-stychnine binding 48K subunit has been cloned and shown to be homologous to other ligand-gated receptor polypeptides (Grenningloh et al., 1987). Furthermore, the 48K polypeptide is capable of assembling into a functional receptor homo-oligomer when expressed in frog oocytes (Schmieden et al., 1989). We report here the isolation of the second component of the glycine receptor core, the 58K subunit. Gas phase microsequencing of 58K tryptic peptides resulted in several amino acid sequences which were used to construct synthetic oligonucleotide probes for screening a λgt10 cDNA library. The deduced amino acid sequence of the isolated cDNA clones indicate strikingly high homology to the 48K subunit. The putative transmembrane regions M1 to M4 and two cysteine residues in the N-terminal hydrophilic domain are conserved. Most divergence occurs in the region connecting M3 and M4. These data show that the glycine receptor channel is composed of homologous subunits similar to nicotinic acetylcholine and $GABA_A$ receptors suggesting that these channels are built on the same structural plan.

GABA AND BENZODIAZEPINE RECEPTORS IV

459.1

PHENYTOIN MODIFIES DIAZEPAM ENHANCEMENT OF GABA-MEDIATED CHLORIDE FLUX. J. Francis, B. Sneddon*, S. Kish*, W.M. Burnham and L. Spero. Dept. of Pharmacology, University of Toronto, Toronto, Ontario and Clarke Institute of Psychiatry, Toronto, Ontario.

We have previously demonstrated that diazepam potentiates specific 3H-phenytoin binding in rat brain [Okazaki, M. et al. Life Sci., 33: 409, 1983]. The current study attempts to investigate, conversely, whether phenytoin interacts with the GABA-A receptor complex or any of its subunits.

GABA-stimulated chloride flux in a synapto-neurosomal preparation from rat cerebral cortex was used. GABA (10 uM) stimulated chloride uptake into synaptoneurosomes by 47% over basal flux. Diazepam (50 uM) had no effect on its own, but potentiated GABA-stimulated chloride flux by 25%. Phenytoin (10 uM) had no effect on basal or GABA-stimulated chloride flux. In the presence of GABA (10 uM) and diazepam (50 uM), however, phenytoin significantly enhanced chloride uptake by 18% ($p<0.05$) (n=4).

Taken together, these data suggest the possibility that, in mammalian brain, the phenytoin recognition site may be allosterically coupled to a benzodiazepine recognition site. These sites, in turn, may be functionally linked with the GABA-A receptor complex.

459.2

STEROID HORMONE METABOLITES POTENTIATE GABA RECEPTOR-MEDIATED CL^- UPTAKE: EVIDENCE FOR MULTIPLE ALLOSTERIC BINDING SITES. J. R. Pace*[1], R. H. Purdy*[2], S. M. Paul*[1], A. L. Morrow[1]. (SPON: L. L. Hsu) [1]Clinical Neuroscience Branch, NIMH, Bethesda, MD 20892 and [2]Southwest Foundation, San Antonio, TX 78284

Endogenous metabolites of progesterone and deoxycorticosterone potentiate GABA-mediated chloride ion flux in rat brain synaptoneurosomes. To investigate the interactions of various natural and synthetic steroids with the GABA-benzodiazepine receptor complex, concentration-response curves were generated for steroid potentiation of muscimol-stimulated $^{36}Cl^-$ uptake and analyzed using the computer program ALLFIT.

Cerebral cortical synaptoneurosomes were prepared from adult male S.D. rats. Muscimol-stimulated $^{36}Cl^-$ uptake was measured for 5 seconds in the presence or absence of steroids. The endogenous steroid metabolites 3α,21-dihydroxy-5α-pregnan-20-one (THDOC) and 3α-hydroxy-5α-pregnan-20-one (3α-OH-DHP) exhibited shallow biphasic concentration response curves with maximal potentiations of 14.5 and 10.9 nmoles $^{36}Cl^-$/mg. protein, respectively. Pseudo-Hill coefficients for THDOC and 3α-OH-DHP- induced potentiation of muscimol-stimulated $^{36}Cl^-$ uptake were 0.46 and 0.56. In contrast, concentration response curves for the synthetic derivative 3α, 21-dihydroxy-5α-pregnan-20-one 21-mesylate were monophasic with an Emax of 9.1 nmoles $^{36}Cl^-$/mg prot and a Hill slope of 0.94. The 3α-hydroxy-5α-androstane-17β-carbonitrile concentration response curves were steep and monophasic with a mean Hill coefficient of 2.2.

These data suggest that steroid hormone metabolites interact with multiple allosteric sites on the GABA-benzodiazepine receptor complex to potentiate GABA-mediated chloride uptake.

459.3

CHRONIC LITHIUM EFFECT ON GABA-MEDIATED SYNAPTONEUROSOMAL CHLORIDE UPTAKE. R.M. SALOMON*, and W.J. SHOEMAKER. Dept. of Psychiatry, Univ. of Connecticut School of Medicine, Farmington, CT 06032.

We have been investigating the role of γ-aminobutyric acid-A ($GABA_A$) receptors in the biological activity of chronic lithium (Li) exposure in the CNS. Using a synaptoneurosome preparation, the functional response following stimulation of the GABA-BZD-chloride channel complex is assessed by uptake of 36-chloride in a 5 second exposure. Cerebral cortical synaptoneurosomes from rats fed a lithium-containing (40 mMol) diet for 3 weeks are significantly more responsive to muscimol stimulation. (Serum Li levels for these animals were 0.90 meq/L.) At 20 μM muscimol, uptake stimulation increased (from control 166% above baseline to 236%) representing a 107% increase in muscimol stimulation ($p<0.05$). Acute Li exposure over a wide physiological range has no effect on muscimol stimulated chloride flux but at supraphysiologic concentrations of lithium an inhibition of Cl- uptake was observed. This may suggest that a chronic lithium effect in mania may be mediated in part by functionally altering GABA-mediated chloride flux. Another observed effect was an enhancement of chloride uptake in the absence of magnesium, which is not reversed by lithium. This work was partially supported by the University of Connecticut Health Center Research Advisory Committee.

459.4

SPECIFIC CHLORIDE INFLUX: RELATIONSHIP BETWEEN $^{36}Cl^-$ UPTAKE RATE AND GABA CONCENTRATION. Ewa Malatynska*, Monique L. Giroux*, Richard S. Jaeckle, Steven C. Dilsaver. Dept. of Psychiatry, Neuroscience Program, The Ohio State Univ., Columbus, OH 43210-1228.

GABA produces a concentration dependent increase of $^{36}Cl^-$ uptake in membrane vesicles prepared from rat cerebral cortex. The transformation of these data as specific chloride influx (SCI), defined as the rate of $^{36}Cl^-$ uptake in nmoles/3 sec/ mg protein/ nomol GABA reveals three distinct chloride conduction states which can be related to the GABA concentration. The first conduction state is characterized by a rapid increase in the SCI over a GABA concentration range of 0-10 μM and reaches a maximum at 10 μM GABA. The second conduction state is defined by a steady SCI value which occurs between 10-30 μM GABA. The third conduction state occurs at GABA concentrations over 30 μM, shows declining values of SCI, and is probably related to the desensitization of the GABA receptor. A fixed concentration (10μM) of the ß-carboline agonist for the BZ-receptor, ZK 93423, shifts to the left the GABA dose-response curve at lower GABA concentrations while depressing the maximal GABA response at higher GABA concentrations.The effects of ZK 93423 on SCI are (1) an increase in SCI value in the first and second conduction states; (2) a change in the second state from steady to very fast declining SCI; (3) a decrease of the SCI in the third state. Thus, the reduction of the maximal GABA response produced by ZK 93423 corresponds to an enhancement of GABA receptor desensitization.

459.5

PYRETHROID INSECTICIDES AND VERATRIDINE INCREASE BASAL CHLORIDE UPTAKE INTO RAINBOW TROUT SYNAPTONEUROSOMES. A.J. Eshleman* and T.F. Murray. Toxicology Program and College of Pharmacy, Oregon State University, Corvallis, Or. 97331.

γ-Aminobutyric acid (GABA)-gated chloride ionophores comprise the main inhibitory neurotransmitter system of the CNS of vertebrates. Insecticides such as endrin and dieldrin have been shown to interact with this receptor/ionophore. Evidence is emerging that pyrethroid insecticides also interact with this system at low micromolar concentrations as measured by effects on ligand binding; a contention exists as to the relevance of this interaction for pyrethroid toxicity as compared to their activity at voltage-gated sodium channels. Rainbow trout (*Oncorhynchus mykiss)* have been used in this study to investigate the ability of pyrethroids to modulate chloride flux due to their sensitivity to these insecticides. Deltamethrin, (1RαS)*cis* cypermethrin and 1R*cis* permethrin have been shown to inhibit 82, 87 and 67% of GABA-dependent ^{36}Cl uptake into trout brain synaptoneurosomes when GABA-dependent uptake is defined as the total uptake in the presence of GABA minus the total uptake in the absence of exogenous GABA, measured at each concentration of pyrethroid. Because these pyrethroids caused a significant increase in basal uptake, we characterized this pyrethroid-dependent uptake: 1. The increase in basal uptake was inhibited by tetrodotoxin (TTX). 2. Veratridine (VTD) caused a greater increase than deltamethrin in basal flux which was also inhibitable by TTX. 3. There was little or no synergy in basal chloride uptake with VTD and deltamethrin. These data suggest that pyrethroids may indirectly increase chloride influx into piscine synaptoneurosomes via a TTX-sensitive effect, most likely at voltage-dependent sodium channels. [Supported by HHS Grant ES04891.]

459.6

EFFECTS OF ATP ON BASAL AND PHENOBARBITAL-ENHANCED SPECIFIC GABA BINDING TO RAT BRAIN MEMBRANES IN VITRO. J.A. Richter and L.R. Curtis*. Indiana Univ. Sch. Med., Indianapolis, IN 46202.

We have shown previously that in an appropriate membrane preparation incubated at 37° in a complete medium, specific ^3H-GABA binding is enhanced not only by pentobarbital (PB) but also by phenobarbital (PheB). In another membrane preparation we and others find better specific binding but no PheB effect.

In the present work we have found that addition of ATP to the binding assay increases specific ^3H-GABA binding and reduces the PheB-enhancement of this binding in our membrane preparation. Preliminary experiments suggest that the ATP effects are also observed with GTP but not with the non-hydrolyzable ATP analog AMPPNP. In experiments to date the ATP effects are not duplicated by 8-bromo-cAMP or inhibited by W-7 (a CaCM kinase inhibitor), Polymixin B (a PK-C inhibitor), or heparin (a casein kinase inhibitor).

These results could suggest that in our membrane preparation there may be an unidentified kinase activity requiring exogenous ATP or GTP. Alternatively there may be an activated phosphatase the effect of which is overcome by the hydrolyzable nucleotides. These suggestions are consistent with the idea that phosphorylation increases GABA binding to high affinity GABA-A receptors and decreases the PheB-enhancing effect. (Supported by grant DA-00796).

459.7

THE INHIBITION OF [3H]-FLUNITRAZEPAM BINDING IN WASHED RAT BRAIN MEMBRANES AND BRAIN TISSUE SECTIONS BY β-CARBOLINES. P.A. Chmielewski* and J.A. Miller (SPON: R.J. Dinerstein), Merrell Dow Research Institute, Cincinnati, OH, 45215.

The radioligand [3H]-flunitrazepam ([3H]-FLU) which recognized both subtypes of the central benzodiazepine receptor (BZD1 and BZD2), was used to label these sites in washed rat brain membranes and sagittal sections. Localization of BZD binding sites was analyzed by quantitative autoradiography. Various β-carbolines were used to displace [3H]-FLU binding and Ki values calculated. The displacement analysis also examined the ability of these agents to distinguish between the two receptor subtypes based on the relative proportion of BZD1 and BZD2 in different brain regions. The BZD antagonist RO15-1788 displayed subnanomolar affinity. The inverse agonist β-carboline 3-hydroxymethyl-β-carboline (3-HMC) had a Ki of 0.5 μM and a Hill coefficient of 1.0. Other β-carboline, however, distinguished between the two receptor subtypes. Methyl-6,7-dimethoxy-4-ethyl-β-carboline-3-carboxylate (DMCM) had Ki's of 8.5 nM and 0.6 nM for BZD1 and BZD2 receptors respectively. The partial inverse agonist, N-methyl-β-carboline-3-carboxamide (FG-7142), was much lower in potency with Ki's of 14 μM (BZD1) and 0.2 μM (BZD2). Ethyl-β-carboline-3-carboxylate (β-CCE) demonstrated a higher affinity for the BZD1 subtype (Ki=0.7 nM) than for BZD2 (Ki=9 nM). These differences in potency and selectivity for the BZD receptor subtypes may explain some of the pharmacological differences among the β-carbolines.

459.8

BIOCHEMICAL EVIDENCE FOR A PARADOXICAL EFFECT ELICITED BY BENZODIAZEPINES. E. Sanna*, A. Concas*, M. Serra* and G. Biggio (SPON: L. Martini). Dept. of Experimental Biology, University of Cagliari, Italy

35S-TBPS binding to membrane preparations from rat cerebral cortex and from cerebellar granule cells in primary culture was used to study the changes in the function of GABAergic synapses induced by GABAergic drugs. Since the action of positive and negative modulators of GABAergic transmission is strictly dependent by the presence of GABA at the synaptic level, we used for this study unwashed membranes which are supposed to be rich of endogenous GABA. In these membrane preparations the "in vitro" addition of diazepam (1 μM) decreased by 46% 35S-TBPS binding to both cerebral cortex and granule cells. This effect was completely reversed by bicuculline (10 μM) which per se enhanced 35S-TBPS binding. Moreover, 35S-TBPS binding was also markedly enhanced by anxiogenic ß-carbolines. The effect of bicuculline was presumably due to the blockade of the inhibitory action of GABA. In fact, the removal of GABA by extensive washes of membranes mimicked the effect of bicuculline and ß-carbolines enhancing 35S-TBPS binding by 90%. Moreover, in well-washed membrane preparations (devoid of GABA), diazepam (1 μM) increased 35S-TBPS binding (+ 23%). Same results were obtained using cerebellar granule cells in primary culture. This finding suggests that in the absence of GABA, diazepam, like bicuculline and anxiogenic ß-carbolines has a negative modulatory action on GABAergic transmission. This conclusion is strongly supported by the result showing that diazepam further enhanced (26%) the bicuculline-induced increase of 35S-TBPS binding to unwashed membrane preparations. The data suggest that the increase of 35S-TBPS binding induced by diazepam in absence of GABA might reflect a paradoxical response similar to some paradoxical behavioural effects elicited by benzodiazepines.

459.9

BUSPIRONE ALTERS BENZODIAZEPINE BINDING BUT NOT GABAa RECEPTOR FUNCTION. F. Lopez,* L.G. Miller, A. Schatzki,* S. Chesley,* D.J. Greenblatt,* (SPON: T. Theoharides). Div. of Clinical Pharmacology, Depts. of Psychiatry and Pharmacology, Tufts-New England Medical Center, Boston, MA 02111.

Buspirone(B), an azospirodecanedione, has recently been introduced as a non-benzodiazepine(BZ) anxiolytic. Since some reports indicate effects of B at the BZ receptor, we assessed effects of B, an analog gepirone(G) and the major metabolite of both compounds, 1-(2-pyrimidinyl)-piperazine (1PP), on BZ binding in vivo and ex vivo and GABA-related chloride uptake ex vivo. BZ binding in vivo was increased in cerebral cortex and cerebellum in mice treated with acute doses of B and G, 5 mg/kg(B5,G5) but not at 1 mg/kg (B1, G1)(CX: Vehicle 1750; B1 1900; B5 2654; G1 1695; G5 2436; all fmol/g). No change was seen with similar doses of 1PP. BZ binding in vitro was unaffected by B and 1PP, but number of sites was increased by G, 5 mg/kg. Muscimol-stimulated chloride uptake was unaffected by B, G or 1PP (Vehicle 24.8; B 25.6; G 25.0; 1PP 25.7; all nmol/mg prot). Thus, buspirone and gepirone may affect BZ binding but do not appear to alter function of the GABAa receptor. The metabolite 1PP does not affect binding or receptor function.

459.10

QUANTITATION OF CELL-SURFACE VS. INTRACELLULAR BENZODIAZEPINE RECEPTORS USING A SULFONATED BENZODIAZEPINE DERIVATIVE. M.H. Jalilian Tehrani and E.M. Barnes, Jr. Dept. of Biochem., Baylor College of Medicine, Houston, TX 77030.

A membrane-impermeant derivative of the benzodiazepine 1012S was prepared by reaction with 4-sulfophenylisothiocyanate and purification by reverse phase HPLC. The resulting N-(4-sulfophenyl)thiocarbamoyl derivative (SPTC-1012S) retained the high affinity of the parent compound for central benzodiazepine receptors on chick cortical neurons in culture. Approximate IC_{50} values for displacement of [^{3}H]flunitrazepam binding to intact neurons were 2 nM and 5 nM for SPTC-1012S and 1012S, respectively. Permeabilization of neuronal surface membranes by exposure of cells to 0.005% saponin had no effect on the level of [^{3}H]flunitrazepam binding or on the ability of 1012S to displace this label. However, [^{3}H]flunitrazepam displacement by SPTC-1012S was increased by 10% following saponin treatment. The data suggest that 90% of cellular benzodiazepine receptors are exposed on the external surfaces of developing neurons. Thus, SPTC-1012S can be used to study the intracellular trafficking of GABA/benzodiazepine receptors.

Supported by NIH grants DK 17436 and NS 11535.

459.11

^{35}S-TBPS BINDING EX VIVO AS AN INDEX OF IN-VIVO GABAERGIC ACTIVITY AND MODULATION. A. Concas*, E. Sanna*, M. Serra* and G. Biggio, Dept. of Experimental Biology, Chair of Pharmacology, University of Cagliari, Italy

The pharmacology of $GABA_A$ receptor was studied by measuring "ex vivo" ^{35}S-TBPS binding in unwashed membrane preparations from the rat cerebral cortex. Anxiogenic and convulsant ß-carboline derivatives produced a dose dependent (0.05-1 mg/kg i.v.) increase of ^{35}S-TBPS binding. On the contrary, benzodiazepines (BZ) and other agonists for BZ receptors markedly decreased ^{35}S-TBPS binding in the same brain area. The effect of anxiogenic and anxiolytic drugs was mimicked by isoniazid (150-600 mg/kg s.c.), an inhibitor of GABAergic transmission, and valproic acid (150-400 mg/kg), respectively. Ro 15-1788, a BZ receptor antagonist, prevented the effects of both anxiolytic and anxiogenic drugs but failed to antagonize the action of isoniazid and valproic acid. Ethanol (1-4 gr/kg) like anxiolytics elicited in 60-90 min. a small but significant decrease of ^{35}S-TBPS binding. The action of ethanol was also studied on both the convulsive pattern and the increase in ^{35}S-TBPS binding elicited by isoniazid. High doses (3-5 gr/kg) of ethanol prevented the convulsions elicited by isoniazid. Consistently ethanol antagonized by 50% isoniazid-induced increase in ^{35}S-TBPS binding. The results suggest that the "ex vivo" measurement of ^{35}S-TBPS is a suitable index of the functional state of $GABA_A$ receptors in vivo.

459.12

SELECTIVE ω_1 (BZ_1) SITE ACTIVATION IS SUFFICIENT TO MODULATE THE $GABA_A$ RECEPTOR-LINKED CHLORIDE IONOPHORE: STUDIES WITH THE HYPNOTIC IMIDAZOPYRIDINE ZOLPIDEM. K.G. Lloyd and F. Thuret*. Biology Dept., Synthélabo Recherche (L.E.R.S.), Paris, 75013, France.

Zolpidem (ZO), is a highly selective ligand for the ω_1 (BZ_1) site in the GABA receptor supramolecular complex (GRSC) (Langer, S.Z. and Arbilla, S. J. Fund. Clin. Pharm., 2: 159, 1988). In order to determine if selective ω_1 site stimulation is sufficient to modulate the GRSC function, we have presently examined the action of ZO at different subunits of the GRSC using ^{35}S-TBPS binding, ^{3}H-GABA binding and $^{36}Cl^-$ uptake. With well-washed rat cerebral cortex membranes in the presence of NaCl, ZO allosterically enhances ^{35}S-TBPS binding (EC_{50}= 80nM, Max. eff.= +36%) to the same extent as GABA (EC_{50}= 388nM, Max. eff.= +31%). This effect is competitively antagonized by flumazenil (1 and 10µM) but bicuculline (1mM) only partially (33%) inhibits the action in a non-competitive manner. The action of GABA is completely antagonized by bicuculline and unaltered by flumazenil. The enhancement of ^{35}S-TBPS binding by ZO appears to be due to an enhanced rate of association of ^{35}S-TBPS. Dissociation of the ligand is also modulated (introduction of a fast component of dissociation) suggesting that ZO increases the frequency of chloride ionophore opening and closing. The findings using ^{35}S-TBPS are confirmed by $^{36}Cl^-$ uptake studies. ZO increases ^{3}H-$GABA_A$ binding to rat cerebral cortex membranes, apparently by enhancing the number of sites available to the ligand. Thus, selective ω_1 site activation by ligands such as ZO is capable of activating the entire sequence of events within the GRSC.

459.13

CHARACTERIZATION AND DISTRIBUTION OF [^{35}S]TBPS BINDING TO RAT BRAIN SECTIONS USING AUTORADIOGRAPHY. Patricia P. Edgar and Rochelle D. Schwartz, Dept. of Pharmacology, Duke University Medical Center, Durham, NC 27710.

[^{35}S]TBPS binding to rat brain sections was characterized for subsequent autoradiographic analysis. Cortical brain mash slices were preincubated with EDTA to remove endogenous GABA. The association and dissociation rate constants were $0.377 min^{-1}pM^{-1}$ and 0.011 min^{-1}, resp. Dissociation was monophasic and slow ($t_{1/2}$=80 min). Scatchard analysis indicated a single population of binding sites (K_D = 21.0 nM, B_{max} = 1.59 pmol/mg prot.). Picrotoxin and muscimol inhibited [^{35}S]TBPS binding with IC_{50}s of 251 nM and 203 nM and n_Hs of 0.98 and 1.4, resp. Distribution of [^{35}S]TBPS binding sites in the rat brain resembles that of other ligands which bind to $GABA_A$ receptor complex with some regionally specific differences. Regions with a high degree of [^{35}S]TBPS binding included inf. colliculus, med. septal n., central thalamic n., olf. tubercle, zona incerta, dentate gyrus, and substantia nigra. [^{35}S]TBPS preferentially bound to the molecular vs granular layer of the cerebellum. Omission of the preincubation or addition of 1 uM GABA to preincubated slices markedly but variably decreased [^{35}S]TBPS binding. For example, [^{35}S]TBPS binding was inhibited to different degrees in the cell layers of the cerebellum. Our study shows that the distribution of [^{35}S]TBPS binding sites is influenced by the preincubation-incubation conditions.

Supported by NS24577 & PMA Foundation.

459.14

DISTRIBUTION OF BENZODIAZEPINE/$GABA_A$ RECEPTORS IN THE MONKEY MEDIAL TEMPORAL LOBE: AN IMMUNOHISTOCHEMICAL ANALYSIS. H. M. Stroessner*, C. R. Houser, J. G. Richards and D. G. Amaral. (SPON: R. Evans) The Salk Institute, La Jolla, CA 92037; UCLA, Los Angeles, CA. 90024; and F. Hoffman-La Roche & Co. Basel, Switzerland

Monoclonal antibodies (bd-17 and bd-24) to the benzodiazepine/$GABA_A$ receptor complex purified from bovine cerebral cortex were used to study the localization of this receptor in immunohistochemical preparations of the macaque monkey amygdaloid complex and hippocampal formation. In the amygdala, the highest density of staining was observed in the lateral and accessory basal nuclei and the nucleus of the lateral olfactory tract. The staining in these nuclei had a particulate appearance and was presumably associated with dendrites. In the basal nucleus and periamygdaloid cortex (PAC), the overall level of particulate staining was low but numerous distinctly labeled neurons were observed. While most of these were small and round with several radiating processes, some larger neurons were also labeled, especially in the PAC. In the hippocampal formation, the densest particulate staining was observed in the molecular layer of the dentate gyrus, within layer II of the presubiculum and throughout the entorhinal cortex except for layer V. Large numbers of neurons were clearly stained throughout the hippocampal formation, particularly in the dentate gyrus and in field CA3 of the hippocampus. In the hippocampus, many of the smaller stained neurons had highly varicose dendrites. Other, larger stained CA3 neurons, however, had smooth dendrites and resembled pyramidal basket cells.

459.15

AUTORADIOGRAPHIC LOCALIZATION OF $GABA_A$ AND $GABA_B$ BINDING SITES IN THE VENTRAL HORN OF THE CAT SPINAL CORD. Debra A. Vascik, Joseph R. Holtman, Jr. and Bruce E. Maley, Dept. of Anatomy and Neurobiology and Dept. of Pharmacology, Univ. of Kentucky Med. Ctr., Lexington, KY 40536.

Gamma-aminobutyric acid (GABA) is considered to be a major inhibitory neurotransmitter in the central nervous system. Our previous studies have demonstrated a uniform distribution of GABA immunoreactive synaptic terminals in the ventral horn of the cat. In the present study we have examined the presence of $GABA_A$ and $GABA_B$ binding sites in cat spinal cord levels C_{4-6} using in vitro autoradiography with [^{3}H]muscimol to label high affinity $GABA_A$ binding sites and [^{3}H]baclofen for $GABA_B$ binding sites. Each ligand was used at a concentration at or below its calculated K_D. Autoradiograms of [^{3}H]muscimol labeled tissue sections demonstrated moderate grain density in a uniform pattern over the entire ventral horn. Although [^{3}H]baclofen labeled tissue sections had an overall lower grain density than $GABA_A$ binding sites, its binding sites were present in the same regions of the ventral horn as the $GABA_A$ binding sites. The distributions of $GABA_A$ and $GABA_B$ binding sites in a pattern similar to GABAergic immunoreactive terminals, suggests a possible morphological correlate for GABAergic receptors in the ventral horn of the cat. Supported by NIH Grant NS23861 to B.E.M.

459.16

AUTORADIOGRAPHIC COMPARISON OF PERIPHERAL-TYPE BENZODIAZEPINE BINDING SITES IN HUMAN AND RAT KIDNEY. J.M. Olson, B.J. Ciliax, W.R. Mancini and A.B. Young. Depts. of Pharmacology and Neurology and the Neuroscience Program, University of Michigan, Ann Arbor, MI 48109.

The affinity of isoquinolines, benzodiazepines and diuretics were compared autoradiographically in postmortem human and rat kidneys. No interspecies differences were observed in the binding of the isoquinoline PK 11195 (Human: K_D = 6.1± 1.5, B_{Max} = 5.3± 1.0 pmol/mg prt.; Rat: K_D = 7.2 nM, B_{Max} = 7.7 pmol/mg prt.). The benzodiazepines Ro5-4864, diazepam and flunitrazepam however, bound with lower affinity to human kidney (K_i = 217± 65 nM, 1.1± 0.9 μM, > 10 μM, respectively) than to rat kidney (K_i = 87± 12 nM, 810± 220 nM, 1.06± .3 μM, respectively). Homogenate studies confirmed the interspecies difference. In both species, the apparent affinity of benzodiazepines for PBBS was significantly higher in homogenate studies than in autoradiographic studies. The ability of some unlabeled diuretics to compete with [^{3}H]PK 11195 also differed between human and rat kidney. The percent of specific [^{3}H]PK 11195 binding which remained in the presence of 1 mM metolazone, diazoxide, hydrochlorothiazide, trichloromethiazide, ethacrynic acid, furosemide and acetazolamide was 7%, 80%, 70%, 100%, 33%, 62%, 82%, respectively in human kidney cortex and was 0%, 81%, 58%, 53%, 62%, 72%, 100%, respectively in rat. Standard errors were 5-10% for furosemide and acetazolamide and less than 5% for all others. Supported by NIH Grant NS 15655.

459.17

AUTORADIOGRAPHY OF LOCUST CNS GABA RECEPTORS. L.P.Schouest,Jr.,T.A.Miller* and R.W.Olsen*. Dept. of Entomol., Univ. of Calif.,Riverside, CA 92521.

Receptor autoradiography was used for the localization and characterization of insect CNS GABA receptors using $GABA_A$ agonists and antagonists as competitive binders to ^{3}H-muscimol and ^{3}H-TBPS. Competitive binding results were compared to sections incubated in ^{3}H-muscimol. Adjacent sections were utilized in the incubation procedure (Lunt, G.G., Robinson, T., Miller, T., Knowles, W.P. and Olsen, R.W. Neurochem. Int. 1985 7 751-754), so differently treated sections were anatomically equivalent. Locust brain and/or thoracic ganglia (*Schistocerca americana*) was dissected out, frozen, sectioned, incubated, dried and exposed to LKB Ultrafilm. Image analysis consisted of enlarging and digitizing the autoradiograms via a linear photodiode array 12-bit CCD camera. Digitized images were transferred to an image analysis program where the quantitated image was edited and analyzed. A four-page image board allowed comparisons between control and three adjacent sections. Tritium standards placed with the slides during the film exposure period provided quantitative calibration of optical density values. Composite images were constructed, stored as a new image thus allowing direct readings of optical density from each autoradiographic image. Optical density readings were automatically converted to DPM based on a standard curve obtained from digitizing calibration standards. Three-dimensional images of brain and thoracic ganglia were compiled from concurrent adjacent sections and transferred into a three-dimensional reconstruction program. Our results indicate competitive binding of GABA receptors was seen for muscimol and TBPS. Overlay subtraction of images showed specific GABA receptor locations for the lamina ganglionaris, some portions of the medulla, lobula plate and glomerulus. GABA receptors were also localized in the thoracic ganglia in specific lateral regions.

459.18

CHARACTERIZATION OF THE BENZODIAZEPINE RADIOLIGAND 125 I-RO 16-0154: POTENTIAL PROBE FOR SPECT BRAIN IMAGING . E.W. Johnson, S.W. Woods, and R.B. Innis. Department of Psychiatry, Yale University School of Medicine, 34 Park Street, New Haven CT 06508.

Benzodiazepine (BZ) receptor function has been implicated in the pathophysiology of several diseases, including epilepsy and anxiety disorders. Brain imaging of neuroreceptors is a noninvasive technique to directly measure in vivo receptor activity. We have examined an iodinated benzodiazepine ligand as a potential probe for Single Photon Emission Computed Tomography (SPECT) brain imaging.

Ro 16-0154 is a close analog of the benzodiazepine antagonist Ro 15-1788, differing only in the substitution of iodine for flourine on the adjacent number 7 carbon. We have used 125 I-labeled Ro 16-0154 in homogenate binding studies prepared from cerebral and cerebellar cortical tissue from both monkey (Macaca mulatta) and human. 125 I-labeled Ro 16-0154 binding was saturable, of high affinity (approx. 2 nM), and had high ratios of specific to nonspecific binding (approx. 40:1). The specificity of the radioligand binding to the BZ receptor was confirmed with multiple displacement studies. Studies are currently ongoing using the 125 I-labeled Ro 16-0154 for both in vivo and in vitro autoradiography of BZ receptors.

These preliminary studies indicate that 123 I-labeled Ro 16-0154 may be a potentially useful SPECT probe for in vivo imaging because of its pharmacological specificity, high affinity, and low nonspecific binding.

We gratefully acknowledge Medi-Physics, Inc. (subsidiary of Hoffman-La Roche, Inc.) for supplying 125 I-Ro 16-0154.

MONOAMINES AND BEHAVIOR IV

460.1

THE ACUTE DOPAMINERGIC ACTIVATING EFFECTS OF NICOTINE ARE REDUCED FOLLOWING REPEATED ADMINISTRATION. P. Vezina, G. Blanc*, J. Glowinski* and J.-P. Tassin*. Chaire de Neuropharmacologie, INSERM U.114, Collège de France, 75231 Paris Cedex 05, France.

Acute systemic injections of nicotine produce increased locomotion in rats and repeating these injections has been shown to produce a more pronounced or enhanced locomotor effect. Different lines of evidence suggest that mesolimbic dopaminergic (DA) activation may underlie the acute locomotor effects of nicotine. The present study assessed the effect of acute as well as repeated systemic injections of nicotine on DA transmission (DOPAC/DA) in different DA terminal fields in the rat.

Acute injections of (-)-nicotine bitartrate (0.4-0.8 mg/kg, base, s.c.) produced substantial increases (41-49%) in the DOPAC/DA ratio in the nucleus accumbens and more moderate increases (26-37%) in the antero-medial striatum. No effect was detected in the dorso-lateral striatum. Surprisingly, repeating these injections reduced or abolished these increases in DA activity: following five daily injections, nicotine produced much reduced increases in DOPAC/DA in the nucleus accumbens (15-26%) and no longer had an effect in the antero-medial striatum.

Given the evidence that enhanced DA transmission underlies behavioral sensitization to psychomotor stimulants and to opiates, the present results suggest that nicotine differs from these two classes of drugs in the manner in which it produces enhanced locomotor effects following repeated injection.

(Supported by NSERC, INSERM and Philip Morris Europe.)

460.2

INDUCTION OF BITING BEHAVIOR (BB) FOLLOWING INTRASTRIATAL INJECTION OF DOPAMINE (DA) AGONISTS INTO RATS WITH UNILATERAL SUPERSENSITIVE DA RECEPTORS. Y. Okamoto*, H. Nakashima* and M. Goldstein (SPON: B. Reisberg). Neurochem. Res. Lab, N.Y. Univ. Med. Ctr., New York, NY 10016.

The role of dopaminergic neuronal systems in behavioral abnormalities of Lesch-Nyhan syndrome and some mental retardation were investigated by measuring the effects of D_1 and D_2 DA agonists on supersensitive DA receptors in rats. Previous studies have shown that microinjections of amphetamine into the middle ventrolateral (MVL) portion of the striatum produce compulsive oral stereotypy (A. Kelley et al., Psychopharmacol. 95:356, 1988). We have now investigated the effects of microinjections of DA agonists into this striatal area of rats with supersensitive DA receptors rendered by unilateral denervation of the nigrostriatal DA neurons with 6 OH-DA. Surgical procedures were carried out under pentobarbital anesthesia and recovery from surgery was monitored. The microinjections of the mixed D_1/D_2 agonist apomorphine, the D_1 DA agonist SKF 38393, or the D_2 DA agonist quinpirole, dose-dependently induce BB for various periods of time. The BB induced by apomorphine is completely prevented by systemic administration of the D_2 DA antagonist raclopride (0.3 mg/kg; i.p.) in combination with the D_1 DA antagonist SCH-23390 (0.3 mg/kg; i.p.), and that induced by SKF 38393 is prevented by SCH 23390 (0.3 mg/kg; i.p.) alone, while that induced by quinpirole is prevented by raclopride (0.3 mg/kg; i.p.) alone. These results indicate that both D_1 and D_2 DA receptors are involved in mediating BB and that the MVL area of the striatum might be one of the centers involved in this abnormal behavior. Supported by NIMH 02717 and NINCDS 06801.

460.3

ENHANCEMENT OF CONDITIONED REINFORCEMENT: EFFECTS OF MICRO-INJECTION OF AMPHETAMINE INTO STRIATAL SUBREGIONS AND OF PEPTIDES INTO VENTRAL TEGMENTAL AREA. A.E. Kelley, J. Delfs* and C.G. Lang*. Dept. of Psychology, Harvard University, Cambridge, MA 02138.

Psychostimulant drugs have been shown to enhance conditioned reinforcement. In this study, hungry rats were trained to associate a compound stimulus (light/click) with delivery of a food pellet. During the test phase,two levers were introduced into the box and pressing the correct lever (CR lever) was reinforced by the stimulus alone. On four test days, amphetamine (0, 0.2, 2.0, 20 ug) was infused into the following regions in separate groups of rats: nucleus accumbens, ventromedial (VMS), ventrolateral (VLS), anterior dorsal (ADS), and posteral dorsal (PDS) striatum. Amphetamine infused into the nucleus accumbens enhanced conditioned reinforcement (CR). Infusion into the VMS selectively enhanced CR at the medium dose, but abolished responding at the high dose. Injection into the VLS produced strongly increased responding which was not selective for the CR lever. Amphetamine infusion into the ADS significantly increased CR only at the highest dose. Infusion into the PDS had no significant effect. In further experiments, substance P (SP) and d-ala-met-enkephalin were injected into the ventral tegmental area (VTA). SP caused a small increase in lever pressing, but this effect was not selective for the CR lever. DALA had no effect on lever pressing.

460.4

FURTHER STUDIES OF THE ROLE OF SUBPALLIDAL-PEDUNCULOPONTINE PROJECTIONS IN LOCOMOTOR ACTIVITY. G. J. Mogenson, M. Wu* & S. M. Brudzynski. Dept. of Physiology & Dept. of Clinical Neurological Sciences, Univ. of Western Ontario, London, Ontario, Canada N6A 5C1.

There is a good deal of experimental evidence implicating nucleus accumbens-subpallidal projections in locomotor activity. It has not been established, however, whether locomotion is mediated by subpallidal outputs to the pedunculopontine nucleus, the site of the mesencephalic locomotor region, or to the mediodorsal thalamus (Mogenson & Wu, Brain Res. Bull., 20:241-246, 1988; Swerdlow & Koob, Brain Res., 412:233-243, 1987). In the present study the role of subpallidal-pedunculopontine projections in locomotor activity was investigated further. Locomotion measured in an open-field was elicited by injecting dopamine unilaterally into the accumbens or by injecting picrotoxin unilaterally into subpallidal region of rats. The effects of unilateral electrolytic lesions or of unilateral injections of kainic acid or ibotenic acid into the pedunculopontine nucleus (PPN) were investigated.

A significant and consistent reduction of locomotor activity was obtained with unilateral electrolytic or kainic acid lesions of PPN. Ibotenic acid lesions of PPN had a smaller or no effect. These results provide an additional evidence that the subpallidal-pedunculopontine output plays an important role in locomotor activity.

460.5

METOCLOPRAMIDE ENHANCES SHOCK-INDUCED FREEZING: IMPLICATIONS FOR DISRUPTION OF AVOIDANCE BY NEUROLEPTICS. J.R. Blackburn and A.G. Phillips, Dept. of Psychology, University of British Columbia, Vancouver, BC, Canada, V6T 1Y7.

Administration of the neuroleptic drug metoclopramide (MET; 5.0 mg/kg) potentiated freezing responses of rats to 1.0 mA footshock, but did not produce any pre-shock freezing. To determine if inappropriate freezing responses could contribute to deficits in active avoidance produced by MET and other neuroleptics, drugged and undrugged rats received unavoidable footshock prior to each of ten one-way avoidance trials, in one experiment, or prior to the avoidance session, in a second experiment. In neither case was the performance of control rats adversely affected, but in each case the performance of MET-treated rats was significantly disrupted. A third experiment demonstrated that presentation of a conditional stimulus previously paired with shock disrupted the avoidance performance of MET-treated rats but enhanced the performance of saline-treated rats. We conclude that the enhancement of freezing by neuroleptic drugs contributes to the deficit in avoidance responding produced by their administration.

460.6

HISTAMINE AND NEUROLEPTIC-INDUCED CATALEPSY. **A.Yagi*, H.Azuma*, T.Nishimura*, T.Yamamoto*§, A.Yamatodani*# and H.Wada*# (Spon: K.Kanosue) Departments of Psychiatry and #Pharmacology II, Faculty of Medicine, Osaka University, Osaka 530 and §Faculty of Liberal Arts, Tezukayama University, Nara 631, Japan.**

The effects of histaminergic neuromodulation on the neuroleptic induced catalepsy was studied in male ddY mice (25-30g). Catalepsy was evaluated by forcing the mouse to grasp a glass bar of 7 mm diameter set horizontally at the height of 4 cm with its forepaws, and recording the duration of the behavior and the response to tail pinching as a score of 0 to 5. Histamine was determined by HPLC-fluorometry. The i.p. administrations of perphenazine (20 mg/kg) and haloperidol (10 mg/kg) induced cataleptic behavior and increases of 29% and 22%, respectively, in the brain histamine content 120 min after their administrations. Dose-dependent changes of brain histamine were observed in the dose ranges of 2 to 20 mg/kg of perphenazine and 2 to 10 mg of haloperidol. Pretreatment with L-histidine (1g/kg, i.p.) enhanced the cataleptic behavior, whereas pretreatment with alpha-fluoromethylhistidine (100 mg/kg), a specific inhibitor of histamine synthesis, reduced it. Furthermore, pretreatments with mepyramine (50 mg/kg, i.p.) and cimetidine (200 mg/kg, i.p.) attenuated the catalepsy. These results suggest that the histaminergic neuron system play some roles in the neuroleptic induced catalepsy.

460.7

LOCAL INJECTION OF DOPAMINE ANTAGONISTS INTO THE PREFRONTAL CORTEX OF MONKEYS INDUCES DEFICITS IN MEMORY-GUIDED SACCADES. T. Sawaguchi and P.S. Goldman-Rakic. Sec. Neuroanat., Yale Univ. Sch. of Med., New Haven, CT06510

While monkeys were performing an oculomotor delayed-response task, dopamine antagonists were injected locally into the prefrontal cortex. Monkeys fixated a central spot, and, following a delay of 1-7 sec, made a memory-guided saccade to the location which had been indicated prior to the delay by a visual cue. Following injection of SCH23390 or haloperidol (10-80ug/1-8ul), the accuracy of saccades to the remembered targets decreased, and the latency of the saccade was increased. These deficits were mainly contralateral to the injection site and always associated with a few specific locations, which varied with injection site. Moreover, the degree of change was dose dependent and also sensitive to the length of the delay period. No significant changes were observed for visually-guided saccades within the same drug sessions when memory-guided saccades were impaired. In addition, injections of sulpiride (up to 100ug/10ul) failed to induce any significant performance decrement either in memory-guided or visually-guided saccades. These results suggest that the activation of dopamine receptors, probably of D1 receptors, is normally involved in the initiation and control of eye movements guided by memory of visuo-spatial representations in the prefrontal cortex.
Supported by MH 44866.

460.8

NONLINEAR SENSITIZATION OF ACTIVITY PRODUCED BY CHRONIC TREATMENT WITH THE D2 AGONIST QUINPIROLE. H. Szechtman, G. Canaran*, F. Ibrahim* and D. Eilam. Dept. Biomedical Sci., McMaster Univ., Hamilton, Ontario, CANADA L8N 3Z5.

An acute injection of quinpirole (0.5-2 mg/kg) has a biphasic effect on activity: in the first minutes after injection, it reduces activity; in the second hour after injection, it increases the amount of forward progression (Eilam et al, BR, in press; EJP 161 (1989) 151). In the present study, we investigated the change in activity produced by chronic treatment with quinpirole (0.5 mg/kg). Rats were tested in a large open field or in Digiscan Activity cages. Results showed that during the course of repeated injections, the profile of progression underwent 4 changes: 1) the period of inhibition diminished across injections, until inhibition was very brief or was not apparent altogether; 2) the onset of excitation advanced progressively, until ultimately excitation began within minutes after injection and did not terminate by the end of a 2 hour test; 3) the speed and the amount of activity increased profoundly, being more than 8 fold higher by end of treatment; and finally, 4) the amount of activity increased non-linearly across injections, rising abruptly after the second or third injection, and then continuing gradually to a new plateau. These data suggest that repeated exposures to quinpirole alter the dynamic properties of dopaminergic systems, shifting them to a new level of responsiveness in discrete steps.

[Supported by The Scottish Rite Schizophrenia Research Program and MRC. HS is a Research Associate of the Ontario Mental Health Foundation].

460.9

COMPULSIVE AND RIGID LOCOMOTION PRODUCED BY CHRONIC TREATMENT WITH THE D2 AGONIST QUINPIROLE. D. Eilam, G. Canaran* and H. Szechtman. Dept. Biomedical Sciences, McMaster Univ., Hamilton, Ontario, CANADA L8N 3Z5.

An acute injection of quinpirole (2 mg/kg) induces hyperactivity, perseverative locomotion along fixed routes, and no stereotypy of movements (Eilam et al, BR, in press). In the present study, we investigated how this pattern is affected by chronic treatment. Rats (n=4) were injected with quinpirole (0.5 mg/kg) and placed in a large open field for 2 hrs for a total of 41 tests. Results showed that during the course of repeated injections, trajectories of progression became increasingly more rigid. Thus, from the 10th injection onwards, as many as 98% of all trajectories were confined to movement along one and the same path. This rigid and compulsive locomotion was rapid and incessant, yielding a very profound elevation in total distance traversed (see previous poster). Marked oral stereotypies or other perseverative movements did not develop. Interestingly, while the behavioural profile to an acute injection varied among the animals, by the end of treatment their behaviour was remarkably alike. Additional tests showed that an injection of saline was insufficient to evoke the drug-induced behaviour, and that the drug-induced profile persisted for at least 2 mo following the cessation of treatment. These results suggest that repeated stimulation of dopaminergic systems produces not only persistent activity (see previous poster) but also confines its spread in the environment to an ever narrower focus. [Supported by Scottish Rite Schizophrenia Research Program. HS is a Research Assoc. of the OMHF].

460.10

Dopaminergic drugs differentially affect attack versus defense in play fighting by juvenile rats. H.M. Marshall*, S.M. Pellis, V.C. Pellis and P. Teitelbaum. Dept. Psychol., Univ. Florida, Gainesville, FL 32611.

Previous studies have reported that low doses of the dopaminergic agonist apomorphine increase the frequency of play fighting in juvenile rats (30-40 days old), whereas low doses of the antagonist haloperidol, decrease the frequency (e.g., Beatty W.W. et al., Pharmacol. Biochem. Behav. 20 : 747-755, 1984). These effects could have occurred via changes in playful attack, playful defense, or both. In the present study, attack (i.e., approach to the nape) and defense (i.e., withdrawal of the nape) were measured separately. After 24 h isolation, 15 min after injection, sibling rats were reunited and their behavior was videotaped under red light for 10 min. Apomorphine (0.05 and 0.3 mg/kg) significantly increased playful attack, whereas haloperidol (0.15 and 0.3 mg/kg) significantly decreased attack. Neither drug affected playful defense at these doses. Therefore, dopaminergic systems affect play fighting via the approach components, not those of withdrawal.

460.11

THE EFFECTS OF DOPAMINE AGONISTS ON THE DRL 72-S SCHEDULE. R. Dunn, D. Jolly* and L. Seiden. Dept. of Pharmacological and Physiological Sciences, Univ. of Chicago, Chicago, IL 60637.

Antidepressants increase the reinforcement rate and decrease the response rate of rats performing on a differential-reinforcement-of-low rate 72-second (DRL 72-S) operant schedule (Seiden et al, Psychopharmacol. **86**:55, 1985). The present work examines the effects of dopaminergic agonists on this antidepressant screen. PPHT, a selective D-2 agonist, decreased the reinforcement rate and increased the response rate of rats performing on a DRL 72-S schedule. Similar DRL 72-S schedule performance changes are seen with amphetamine. In contrast, up to 10 mg/kg of the D-1 agonist SKF 38393 did not significantly affect DRL 72-S schedule performance. These results are consistant with the effects of amphetamine on the DRL 72-S schedule being mediated by D-2 agonist activity. Low doses of apomorphine increased the reinforcement rate and decreased the response rate of rats on a DRL 72-S schedule. Low doses of apomorphine may have preferential activity at dopamine autoreceptors. The dopamine autoreceptor agonists TL-99 and 3-PPP also increased the reinforcement rate and decreased the response rate of rats on a DRL 72-S schedule. Thus, dopamine autoreceptor stimulation induced antidepressant-like effects on the DRL 72-S schedule. This research was supported by PHS MH-11191; RSA MH-10562 (L.Seiden).

460.12

INDUCTION OF SELF-INFLICTING BITING BEHAVIOR (SBB) FOLLOWING INTRASTRIATAL INJECTION OF DOPAMINE (DA) AGONISTS INTO RATS WITH UNILATERAL SUPERSENSITIVE DA RECEPTORS. Y. Okamoto*, H. Nakashima* and M. Goldstein (SPON: B. Reisberg). Neurochem. Res. Lab, N.Y. Univ. Med. Ctr., New York, NY 10016.

Since microinjections of amphetamine into the middle ventrolateral (MVL) portion of the striatum produce compulsive oral stereotypy (A. Kelley et al., Psychopharmacol. 95:356, 1988), we have investigated the effects of microinjections of DA agonists into this striatal area of rats with supersensitive DA receptors rendered by unilateral denervation of the nigrostriatal DA neurons with 6 OH-DA. The microinjections of the mixed D_1/D_2 agonist apomorphine, the D_1 DA agonist SKF 38393, or the D_2 DA agonist quinpirole, dose-dependently induce SBB for various periods of time. The SBB induced by apomorphine is completely prevented by systemic administration of the D_2 DA antagonist raclopride (0.3 mg/kg) in combination with the D_1 DA antagonist SCH-23390 (0.3 mg/kg), and that induced by SKF 38393 is prevented by SCH 23390 (0.3 mg/kg) alone, while that induced by quinpirole is prevented by raclopride (0.3 mg/kg) alone. These results indicate that both D_1 and D_2 DA receptors are involved in mediating SBB and that the MVL area of the striatum might be one of the centers involved in this abnormal behavior. Supported by NIMH 02717 and NINCDS 06801.

460.13

THE EFFECTS OF APOMORPHINE ON ACTIVITY AND EXPLORATION OF RATS WITH FORNIX TRANSECTIONS. S. Wood* and B. Osborne. Department of Psychology, Middlebury College, Middlebury, VT 05753.

Previously we have demonstrated that selective dopamine agonists reduce the activity levels of rats with fornix transections back to control levels. The present work examines the possibility that the agonists are increasing dopamine by acting selectively on autoreceptors. Control and fornix lesioned rats were assigned to 3 groups and received a low dose of apomorphine, a high dose of apomorphine, or a vehicle injection. Ten minutes after the injection, the rats were placed in an open field environment for ten minutes. A detailed behavioral analysis was performed that noted total activity as well as latency to move, and number and duration of rearing bouts, object interactions, and stereotypy. Previous findings were replicated including an increase in total activity and number of object interactions with decreased durations for fornix lesioned animals. Apomorphine produced a general depression of total activity for all groups as well as increased object durations with fewer object interaction bouts. The control animals displayed more stereotyped behavior than the fornix animals. Interpretations of the data are difficult because of the strong effect of apomorphine on control animals. Either the dose levels of apomorphine were too high or previous reports are not due to stimulating autoreceptors.

460.14

GENETIC SELECTION FOR AGGRESSION IN MICE: REVERSAL BY LOW–DOSE APOMORPHINE. M.H. Lewis, J.L. Gariepy, L.L. Devaud, S. Southerland, R.B. Mailman, and R.B. Cairns*. Biol. Sci. Res. Ctr. and Depts. of Psychiatry, Pharmacology, and Psychology, University of North Carolina, Chapel Hill, NC 27514

ICR mice have been selectively bred over 19 generations for high or low levels of aggression. Low aggressive mice have decreased concentrations of dopamine, DOPAC, and HVA in nucleus accumbens and caudate nucleus, accompanied by an increased dopamine receptor density in these same terminal regions. The hypothesis that central dopamine systems mediate genetic selection effects was tested further in the present study. High (NC900) and low (NC100) aggressive lines of mice were treated with apomorphine (0.01–0.55 mg/kg), and their non–social and social behavior quantified. Apomorphine (ED_{50}=0.1 mg/kg) dramatically altered the behavior of the high aggressive (NC900) line, eliminating attack behavior and inducing freezing, behaviors characteristic of the low aggressive (NC100) line. Apomorphine also significantly altered the frequency of non–social behavior, decreasing jumping, climbing, rearing, and locomotion. These effects were not due simply to impairment of motor activity, as apomorphine significantly increased the frequency of active avoidance of the test partner, startle responses, reflexive kicking, and freezing. Low aggressive (NC100) mice also showed decreased attack behavior, and increased avoidance, startle, and reflexive kicking, as well as decreases in climbing, rearing, and locomotion. Neurochemical analyses showed that the behavioral effects were related to drug–induced decreases in the synthesis and release of dopamine. These data support the hypothesis that dopamine mediates line differences induced by genetic selection. (Supported, in part, by PHS Grants HD14648, HD03110, and HD07201.)

460.15

ASYMMETRICAL DOSE-RESPONSE FOLLOWING MICROINJECTIONS OF APOMORPHINE IN THE NUCLEUS ACCUMBENS. M.Honig*, I.Belcheva* S.E.Starkstein, T.H.Moran, R.G.Robinson

In previous studies, we reported that right but not left hemisphere electrolytic lesions of the nucleus accumbens (NA) produced a sustained (30 + day) period of spontaneous running wheel hyperactivity (Kubos, K.L. et al., Brain Res. 401:147-151,1987) and that right but not left fronto-lateral cortical suction lesions produced an increased turnover of dopamine in the NA (Starkstein, S.E. et al., Brain Res., 473:74-80,1988). In the present experiment, male Sprague-Dawley rats (N=9) were implanted with chronic bilateral cannulas in the NA and randomly given right or left NA injections of .32, 1, 3.2, or 10 ug of apomorphine or saline in 1 ul volume every 3 days. Post-injection activity was monitored in 20 minute intervals over 2 hours using computerized photocell (Omnitech) chambers. A 3-way ANOVA revealed significant effects of hemisphere of injection (F_1, 480 = 12.1, $p<.001$), dose (F_4, 480 = 12.7, $p<.001$) and time (F_5, 480 = 36.1, $p<.001$). The only interaction that was significant was side by dose (F_4, 480 = 5.2, $p<.001$). Post-hoc planned comparison t-tests demonstrated significant right versus left hemisphere differences over the 2 hour test period for the .32 ug and 1.0 ug doses (t-12.8 and 8.0, $p<.001$ respectively). These data are consistent with our previous suggestion that subcortical asymmetries in the NA or at another post-synaptic site may play an important role in spontaneous activity and the asymmetrical response to cortical or NA lesions.

460.16

DOPAMINERGIC EFFECTS ON MATERNAL BEHAVIOR AND MILK-EJECTION IN LACTATING RATS. J.M. Stern and L.A. Taylor
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Dopamine (DA) is important in the maintenance of repetitive, stereotyped motor sequences. Moderate doses of haloperidol (HAL), a non-selective dopamine antagonist, disrupt the motorically active components of female sexual behavior (hops, darts; i.e., proceptivity), while leaving the lordosis reflex (i.e., receptivity) intact (Hansen, Stanfield, & Everitt, Neuroscience, 1981). Accordingly, we tested whether a similar dissociation occurs between the motorically active components of maternal behavior (retrieval, licking) and nursing behavior (posture characterized by immobility, a pronounced dorsal arch, and splayed legs). In addition, we re-examined the controversial issue of whether DA inhibits or stimulates oxytocin release, and therefore milk-ejection.

On day 7 or 8 postpartum, dams were separated from their litter for 4 hours. One hour prior to reunion, dams were injected i.p. with HAL (0, 200 ug, 400 ug, 5 mg, or 8 mg/kg). At five minutes after reunion, the experimentor gathered pups in the nest and placed the dam over them, if necessary.

We found a dose-dependent disruption of retrieval and licking of pups. Nursing behavior, however, was not impaired; rather, the crouching posture was enhanced. At 30 minutes after the onset of nipple attachment (by 6 out of 8 pups), the 200 and 400 ug litters gained about 4 times as much weight as controls; 5 mg litters gained about 2 times as much, while 8 mg litters did not differ from controls.

These data suggest that normally DA is necessary for the motorically active components of maternal behavior and inhibits milk-ejection (i.e., oxytocin release). Thus, nursing behavior and physiology depend on inhibition of DA, within physiological limits.

(Supported by NIMH Grant MH 40459.)

460.17

D1 AND D2 RECEPTORS IN THE MPOA REGULATE COPULATION AND PENILE REFLEXES IN MALE RATS. E. Hull, R. Eaton*, J. Thompson*, T. Bazzett* and V. Markowski*.
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We have reported that the D2 agonist LY-163502 (quinelorane) or the D1 antagonist SCH-23390, microinjected into the medial preoptic area (MPOA) of male rats, delayed the onset of copulation and slowed its rate, but then lowered the ejaculatory threshold (decreased the number of intromissions required to trigger ejaculation). We now report that the D2 agonist also decreased the number of erections and penile movements in reflex tests of restrained, supine animals. Seminal emissions were increased. Thus, the delayed onset and slowing of copulation may have reflected an impairment in erectile function, which is primarily controlled by the parasympathetic system. The decreased ejaculatory threshold in copula may have been associated with an increase in sympathetically mediated seminal emission.

The D2 antagonist raclopride in the MPOA also delayed the onset of copulation and decreased its rate, and lowered ejaculatory threshold, similar to the effects of the D2 agonist. Preliminary data suggest that its effects on penile reflexes are also similar to those of the D2 agonist. Thus, it appears that any shift in the D1/D2 ratio in the MPOA may alter the balance of autonomic influence on reflexive penile mechanisms, and thereby alter copulatory parameters.

Supported by NIMH grant #40826.

460.18

DOPAMINE-2 AGONIST, LY163502, ACTS CENTRALLY TO STIMULATE MALE SEX BEHAVIOR OF RHESUS MONKEYS. S. M. Pomerantz. Dept. of Physiology, Univ. of Pittsburgh Sch. of Med., Pittsburgh, PA 15261.

Recent studies demonstrated that dopamine agonists facilitate male sexual behavior of rhesus monkeys (Soc. Neurosci. Abstr. 14: 664, 808, 1988). In order to determine whether the D2 agonist, LY163502, acts centrally or peripherally to stimulate male sexual behavior, the ability of dopamine antagonists, domperidone (DOM, peripheral action only) or haloperidol (HAL, central and peripheral action) to block the sexual response to LY163502 was examined. Non-copulatory male sex behaviors of monkeys (penile erection and masturbation) were assessed in a testing situation in which monkeys were presented with a female monkey they could see, hear and smell, but not physically contact. Eight monkeys were rotated through treatments in which they recived either 50, 100 or 200 µg/kg DOM or vehicle forty min prior to testing and 5 µg/kg LY163502 or vehicle 10 min prior to testing. LY163502 facilitated penile erection ($p<0.001$) and masturbation ($p<0.05$) over vehicle levels, both in monkeys pretreated with DOM and those not pretreated with DOM. Evidence that DOM was acting as a peripheral dopamine antagonist was obtained from two male monkeys in which 100 µg/kg DOM markedly stimulated prolactin release. Administration of LY163502 following DOM in these monkeys failed to reduce prolactin to control baseline levels. In contrast to DOM, the centrally active dopamine antagonist, HAL, blocked the sexual response to LY163502. In monkeys pretreated with 10 µg/kg HAL 50 min prior to LY163502, male sexual behavior was markedly reduced compared to monkeys pretreated with vehicle prior to LY163502. These results indicate that systemically administered dopamine agonists act centrally to stimulate male sexual behavior of rhesus monkeys.

460.19

HALOPERIDOL SUPPRESSES LEVER PRESSING FOR FOOD BUT INCREASES FREE FOOD CONSUMPTION IN A NOVEL FOOD CHOICE PROCEDURE. J.D. Salamone, L.D. McCullough, and R. Steinpreis (SPON: W. Wilson).
Department of Psychology, University of Connecticut Storrs, CT 06269-1020.

An aspect of motivation that is related to motor function is that organisms will perform work to obtain access to significant stimuli. A novel procedure was developed to investigate how this phenomenon is related to dopamine function. Food-deprived rats were tested in an operant chamber in which they could press a lever to obtain a more-preferred food, or freely feed on a less-preferred food. Untreated rats pressed the lever to obtain the more-preferred food pellets, and ate little of the less-preferred food even though it was freely available. Injection of 0.1 mg/kg of haloperidol (HP) dramatically shifted the behavior of these rats, such that the number of lever presses was reduced, but the consumption of the less-preferred food increased significantly. Thus, a low dose of HP suppressed lever pressing for food, although the behavior of the animal still was directed towards food consumption. The present results may be related to the motor slowing that occurs in mild Parkinsonism and depression.

460.20

HALOPERIDOL IMPAIRS CLASSICALLY CONDITIONED EYELID RESPONSES AND CONDITIONING-RELATED ACTIVITY IN THE INTERPOSITUS NUCLEUS. L.L. Sears* and J.E. Steinmetz, Prog. in Neural Sci., Dept. of Psych., Indiana Univ., Bloomington, IN, 47405.

Previous studies of the effects of haloperidol (HAL) on a form of motor learning, classical conditioning of the rabbit eyelid response, showed a decrease in the number of conditioned responses (CRs) when intermediate intensities of an acoustic conditioned stimulus (CS) were used suggesting that the neuroleptic increased the threshold for detecting an auditory CS (Harvey, J.A. & Gormezano, I., J. Pharmacol. Exp. Therap., 218:712, 1981). In an initial attempt to describe the effects of HAL on neural activity associated with eyelid conditioning, the present study examined the effects of HAL administration on learning-related activity in the cerebellar interpositus nucleus, a structure known to be involved critically in classical eyelid conditioning.

While under ketamine/xylazine anesthesia, 8 rabbits were implanted with multiple-unit recording electrodes in the left interpositus. Following recovery, each rabbit was given 6 classical eyelid conditioning sessions with a tone CS and air puff US (12 blocks/session, 10 trials/block) while interpositus recordings were made. Four CS intensities were used during training (95, 85, 75, and 65 dB). Before Sessions 1-3, saline was injected as a control procedure. During Sessions 4-6, 4 blocks of training were given (1 block at each CS intensity), 250 µg/kg HAL was injected i.v., 10 min allowed to elapse, and 8 blocks of post-drug paired training then given. No differences in spontaneous activity of the interpositus were observed when pre- and post-HAL data were compared. No behavioral deficits or changes in interpositus firing patterns were observed when the 95 dB tone was used. However, a significant decrease in percent CRs and a significant reduction in learning-related interpositus activity were observed when 85 and 75 dB tones were used. Training with a 65 dB tone failed to produce CRs or training-related interpositus activity. These data indicate that in addition to disrupting CRs, HAL also disrupts CR-related activity in the interpositus nucleus when intermediate intensities of an acoustic CS are used. HAL apparently disrupts conditioning by altering interpositus activity directly or by altering activity in brain regions afferent to the interpositus nucleus. [Supported by NIMH Grant #MH44052.]

461.1

L-2-AMINO-4-PHOSPHONOBUTYRATE REDUCES CALCIUM CURRENTS IN HIPPOCAMPAL CA1 AND ENTORHINAL CORTICAL NEURONS MAINTAINED IN CULTURE.

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The depression of synaptic transmission by the glutamate analog L-2-amino-4-phosphonobutyrate (L-APB) in certain hippocampal pathways is thought to occur presynaptically. Whole-cell recordings were obtained from neonatal rat hippocampal CA1 and entorhinal cortical neurons maintained in culture for 4-14 days. Sodium currents were blocked by TTX (0.5 μM) and outward currents by internal cesium (150 mM). ATP (5 mM), to reduce run-down of calcium currents, and GTP (1 mM) were included in the internal solution. In the presence of calcium (2mM) or barium (2mM) depolarizing steps from -70 and -40 mV produced inward currents that were completely blocked by external cadmium chloride (200 μM). L-APB (10-100 μM), applied by fast-perfusion, rapidly and reversibly reduced the peak calcium current by 5-25 % in 16/23 neurons examined. L-glutamate (1 μM) mimicked the action of L-APB under conditions in which activation of NMDA, kainate and quisqualate receptors was prevented by kynurenic acid (200 μM), D-2-amino-5-phosphonovalerate (20 μM) and 6-cyano-7-nitroquinoxalone-2,3-dione (20 μM). A reduction in calcium entry into presynaptic neurons may explain the depression of synaptic transmission induced by L-APB.

Supported by NIH grant NS21419 and the McKnight Foundation

461.2

INTERACTIONS BETWEEN QUISQUALATE/KAINATE AGONISTS AT EXCITATORY AMINO ACID RECEPTORS ON STRIATAL NEURONS IN PRIMARY CULTURE.

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We voltage-clamped striatal neurons (cultured 7-8 days from E15 mice) at -80mV (with KF patch-electrode) to examine interactions among the following excitatory amino acid agonists: kainate (KA), quisqualate (QA), glutamate (Glu) and AMPA (a selective QA agonist). Each agonist was applied either by pressure ejection from a micropipette, or by bath perfusion. In most cells, a sustained inward current (which reversed near 0mV) was activated by each of the agonists (10-100μM); at equimolar concentrations the relative amplitude of these currents was in the order of: KA >> Glu = AMPA > QA. When 100μM QA was applied to some cells by pressure ejection, a transient inward current was also activated; similar transient currents were never observed with pressure ejection of KA, at concentrations up to 10mM. In some cells, only a KA-activated current was observed; application of 100μM Glu, AMPA or QA on these cells did not induce a detectable inward current. In all cells, the current activated by pressure ejection of KA (10μM-10mM) was significantly reduced by bath application of 100μM QA, but not by Glu or AMPA. Identical results were observed when the methods of application were reversed. The fractional reduction in KA-activated current by 100μM QA was approximately constant for 10μM-10mM KA, suggesting that the interaction between QA and KA was not competitive. Our data suggest that cultured striatal neurons express at least two types of QA/KA receptors, one of which is selectively activated by KA; however, all QA/KA receptors are selectively inactivated or desensitized by 100μM QA. In addition, these data support the hypothesis (see Weiss and Bauce, Soc. Neurosci. Abs., this volume) that multiple receptor systems subserve specific roles in the regulation of striatal neuron function by excitatory amino acids.

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

461.3

KAINIC ACID-INDUCED SEIZURES: EFFECTS OF ANTICONVULSANTS AND NIFEDIPINE.

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The quisqualate and kainic acid excitatory amino acid receptors are related, and antagonists of the kainic acid and quisqualate receptors have recently been identified (Science 241:701, 1988). Depakene and glutamic acid diethyl ester (GDEE) inhibit quisqualate-induced seizures. The purpose of the present study was to evaluate effects of anticonvulsant drugs and other compounds on kainic acid-induced seizures. A 1 μg intracerebroventricular injection of kainic acid produced seizures in 100% of 18 adult female Swiss- Webster mice lasting for an average duration of 35 minutes. Phenobarbital, baclofen, phenytoin, GDEE, and carbamazepine had no effect. Depakene, at dosages of 125 and 250 mg/kg, prolonged the average seizure duration to 54 and 59 minutes respectively. Diazepam in sedating dosages (2 mg/kg) reduced seizure duration (mean= 18 min). The calcium channel inhibitor nifedipine was the only agent found to markedly decrease these seizures. A dosage of 50 mg/kg produced about a 50% decrease, primarily as a result of decreases in the length of individual seizure episodes. Thus there are substantial differences in the antagonism of quisqualate and kainic acid-induced seizures. The ineffectiveness of clinical anticonvulsants in this model could be related to the unresponsiveness of certain intractable seizure patients.

461.4

HYDROGEN ION INHIBITS THE QUISQUALATE/KAINATE RESPONSE BY PROTONATION OF HISTIDINE GROUPS IN ISOLATED CATFISH HORIZONTAL CELLS.

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The whole cell patch technique was used to measure kainate (KA) or quisqualate (QA) induced membrane current from enzymatically dissociated catfish cone horizontal cells. Increasing the external hydrogen ion concentration reversibly decreased the agonist induced membrane current. The pK was estimated from a titration curve in which the membrane current was measured as a function of pH in a constant concentration of agonist. The pK was 6.5 which is near the pK for the imidazole ring of the amino acid histidine. To examine further the possibility that the amino acid histidine is involved with the ion permeation process at this channel protein we examined the action of the histidine group specific reagent diethylpyrocarbonate (DEP). DEP (1 mM) reduced the response to KA or QA by as much as 50% following a 5 min exposure at either pH 6.5 or pH 8.0. The specific sulfhydryl reagent iodoacetic acid (10 mM) had no effect on the agonist induced membrane current. These results suggest that the imidazole ring of the histidine amino acid is associated with the ion permeation process of the QA/KA channel protein. Supported by grant EY-01897 from the Department of Health and Human Services.

461.5

CURRENT GATED BY KAINATE IN RAT DORSAL ROOT GANGLION NEURONS: CON A ABOLISHES DESENSITIZATION.

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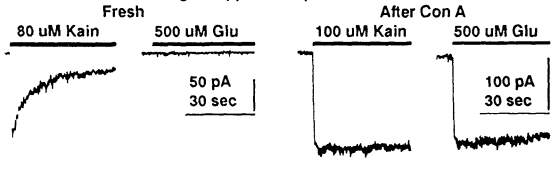
Primary afferent C fibers in rat dorsal roots are depolarized by the excitatory amino acid, kainate (Agrawal and Evans (1986) Br. J. Pharm. 87:345). Under whole-cell voltage clamp, Kain and domoate increase the conductance of a subpopulation of small diameter (<35μm) sensory neurons, freshly dissociated from rat DRG's. The I-V is linear from -100 to +60 mV and reverses near 0 mV with internal Cs^+ or K^+ and internal Cl^- or $CH_3SO_3^-$ (NaCl + 2 mM $CaCl_2$, HEPES and TTX, external). The half-maximal dose is 15 μM for Kain and 0.8 μM for Dom. The currents desensitize with long agonist applications; decay is more rapid with Kain (τ-8 sec) than Dom (τ-18 sec). Kynurenate, PDA and DNQX block responses to Kain and Dom with rapid onset and recovery. Glu, Quis, Asp and NMDA do not evoke current, but simultaneous application of Glu or Quis antagonizes responses to Kain or Dom. Exposure to Glu or Quis also suppresses responses to a subsequent application of Kain or Dom ($t_{1/2}$ of recovery=2-3 min). Incubation with 10 μM Con A abolishes desensitization to Kain and Dom. After Con A, both Glu and Quis increase membrane conductance and no longer suppress responses to Kain or Dom.



461.6

PHARMACOLOGICAL CHARACTERIZATION OF A KAINATE (KA) RECEPTOR EXPRESSED IN *XENOPUS LAEVIS* OOCYTES INJECTED WITH RAT BRAIN mRNA.

M. A. Bolanowski*#, G. B. Watson, M. P. Baganoff*#, C. L. Deppeler*#, and T. H. Lanthorn, CNS Disease Research, G. D. Searle & Company, and #Biological Sciences, Monsanto Company, 700 Chesterfield Village Parkway, St. Louis, MO 63198.

Xenopus laevis oocytes express a wide variety of biological activities encoded by exogenous mRNA, including numerous receptors and ion channels. Kainate elicits an inward current in oocytes injected with rat brain mRNA, indicating the expression of a kainate sensitive receptor/channel complex. We have begun to characterize this functional kainate receptor in order to compare it to physiologically relevant kainate receptors. Voltage clamped oocytes responded to KA in a dose dependent manner (EC50=91 μM). These responses were antagonized by quisqualate (QA; IC50=1-10 μM), 6,7-dinitro-quinoxaline (DNQX; IC50=0.3-0.4 μM), and kynurenate (KYNA; IC50=90 μM). The N-methyl-D-aspartate (NMDA) receptor antagonist D-2-amino-7-phosphonoheptanoate (D-AP7) did not block responses to KA. The kainate analog domoate (DOM) was more potent (EC50=10 μM) than KA. DOM responses were blocked by QA and DNQX at concentrations similar to those which blocked KA responses. In addition, we have tested the activities of other KA analogs, including ß-kainate and dihydrokainate, as well as several non-NMDA agonists (e.g. willardiine and 5-bromo-willardiine). These characteristics demonstrate the presence of a low affinity KA receptor in oocytes injected with rat brain mRNA.

461.7

QUISQUALIC ACID MEDIATED INTRACELLULAR CALCIUM MOBILIZATION IS INHIBITED BY PHORBOL ESTERS BUT NOT BY PERTUSSIS TOXIN. Shawn N. Murphy*, James A. Holzwarth*, and Richard J. Miller, (SPON:W.J.Kinnier) Univ. of Chicago, Chicago, IL 60637.

We have previously demonstrated using fura-2 based microspectrofluorimetry that mouse hippocampal neurons in monolayer culture posses a glutamate receptor whose activation leads to mobilization of Ca^{2+} from intracellular stores and does not appear to be linked to a cation channel. In addition to glutamate, quisqualate (QA) and ibotenate activated this receptor but not α-amino-3-hydroxy-5-methylisoxazole proponate (AMPA). Furthermore the stimulatory effects of glutamate and QA were not blocked by 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX). We have now further investigated the nature of the transduction mechanism involved in this process. Treatment of the neurons with 2.5 μg pertussis toxin (PTX) for 48 hours had no effect upon QA stimulated Ca^{2+} mobilization. Carbachol (CCh) and phenylephrine stimulated Ca^{2+} mobilization were also unaffected by PTX pretreatment although 2-chloro-adenosine inhibition of Ca^{2+} currents was reduced by PTX pretreatment (see Scholz et al. this volume). Treatment of the neurons with 1 μM phorbol 12,13-dibutyrate for 30 minutes prior to agonist application reduced the QA and CCh mediated Ca^{2+} mobilization by > 90%. Thus it appears that the pathway mediating QA induced Ca^{2+} mobilization may be subjected to an inhibitory protein-kinase C feedback as are many other systems involved in inositol-1,4,5-trisphosphate production.

461.8

EFFECTS OF QUISQUALATE AND NMDA ON PHOSPHATIDYLINOSITOL TURNOVER IN RAT CEREBELLUM. Joanna Feltham* and Sheryl S. Smith, Dept. of Anatomy, Hahnemann Univ., Phila., PA 19102. (SPON: Carlos Jimenez-Rivera).

We have previously shown that the excitatory amino acids (eaa) Quisqualate (QUIS) and NMDA produce long-term synergistic effects on QUIS-evoked excitatory responses of cerebellar Puirkinje cells recorded in vivo. In an attempt to delineate a mechanism for these findings, we have assessed eaa-stimulated phosphatidylinositol (PI) turnover in the present study, as well as the effect of possible modulating factors on this parameter. PI hydrolysis was measured in 160 x 160 μ chunks of cerebellar tissue from 6-9 day old female Long-Evans rats. Uptake of [^{3}H] myo-inositol took place in the presence of 1 mM EDTA, and the subsequent hydrolysis of PI in the presence of 3 mM $CaCl_2$. Inositol phosphate and PI were then separated by chloroform/MeOH extraction, and the PI turnover rate assessed as a percentage of basal under varying conditions. 10^{-6} M QUIS routinely produced robust increases in PI turnover as a dose-dependent effect, as shown by other investigators. Addition of NMDA (10^{-4} M) enhanced the ability of QUIS to increase PI turnover by 70% in the presence of the $GABA_B$ blocker phaclofen (10^{-3} M). Phaclofen alone decreased basal PI turnover by 20% and had no effect on QUIS stimulated values. However, administration of bicuculline methiodide exerted only minimal effects on this parameter. In addition, pulsatile application of QUIS (3 pulses at 3 x 10^{-7} M) produced a 70% increase in PI turnover above basal values in the presence of phaclofen, indicative of a QUIS-priming effect similar to that observed after multiple iontophoretic pulses of this amino acid. In the non-blocked condition, pulsatile application of QUIS produced a PI turnover rate 30% above basal, a value similar to that obtained with a single dose of QUIS. In contrast, 17 β estradiol (10^{-9} M) decreased QUIS-evoked PI turnover by 20%. Other reports have indicated that GABA blockers permit the induction of LTP in the cortex. These findings are consistent with the present results suggesting that under conditions of reduced GABAergic tone, the eaa QUIS and NMDA produce synergistic effects on the turnover of PI, a second messenger system which may lead to long-term changes in neuronal function in the developing cerebellum. *Supported by NS25809 to SSS.*

461.9

DIFFERENTIAL EFFECTS OF EXCITATORY AMINO ACIDS ON CYCLIC AMP ACCUMULATION IN RAT BRAIN SLICES. A. Pilc, W. Karbon*, J. Ferkany, J. Vetulani*, S.J. Enna. Institute of Pharmacology, Polish Academy of Sciences, 31-343 Krakow, Poland; Nova Pharmaceutical Corp., Baltimore, MD 21224-2788.

The effects of excitatory amino acids (EAA) on cAMP accumulation in rat brain cerebral cortical slices were measured using a prelabeling technique. Glutamate (0.1-5.0 mM), which by itself caused a slight increase in basal cAMP accumulation, inhibited up to 60% the cAMP response to NE, but not isoproterenol, in a concentration-dependent manner, suggesting that it modifies the alpha component of the NE response. The inhibitory effect of glutamate was mimicked by quisqualate (0.01-1.0 mM), but not by NMDA or kainate, at concentrations up to 5 mM. The inhibitory effect of glutamate was insensitive to CPP (0.10 mM), supporting the notion that the glutamate effect is not mediated by NMDA receptors. In contrast to glutamate, ibotenate (0.01-1.0 mM), which alone caused a modest increase in basal cAMP levels, enhanced the cAMP response to NE more than 5-fold.

These findings provide an additional mechanism by which EAA can influence second messenger production in brain, and the data suggests that a quisqualate receptor mediates the inhibitory effect of glutamate on NE-stimulated cAMP accumulation.

461.10

KAINATE EVOKES THE RELEASE OF ENDOGENOUS GLYCINE FROM STRIATAL NEURONS IN PRIMARY CULTURE. S. Weiss and L. Bauce*. Neuroscience Research Group, University of Calgary, Calgary, Alberta T2N 4N1.

The actions of selective excitatory amino acid (EAA) agonists and other depolarizing agents on the release of endogenous glycine and GABA from striatal neurons in primary culture was examined. The concentrations of endogenous amino acids released into the extracellular medium was determined by pre-column derivitization and separation with high performance liquid chromatography. Baseline levels of glycine and GABA, released by striatal neurons into the extracellular medium during a 3 min period, were 0.73±0.07μM and 0.09±0.02μM, respectively. When striatal neurons were exposed to 56mM KCl for a 3 min period, glycine and GABA levels increased to 1.11±0.10μM (1.5-fold) and 1.96±0.11μM (28-fold), respectively; 50% of these increases were dependent upon the presence of extracellular calcium. Exposure of striatal neurons to NMDA (100μM-1mM) increased glycine and GABA released to 1.11±0.16μM (1.5-fold) and 0.22±0.02μM (2.4-fold), respectively. Kainate was the most effective agent (of those tested) in evoking the release of endogenous glycine. After exposure of striatal neurons to 1mM kainate for 3 min, extracellular glycine levels were increased to 1.72±0.15μM, corresponding to a 2.4-fold increase over baseline; GABA levels were increased 4-fold. This action of kainate was independent of extracellular calcium and was blocked by co-incubation with 3μM CNQX, a selective kainate receptor antagonist. The kainate evoked increases in endogenous glycine release, to 1.72μM, correspond to those concentrations of glycine that significantly potentiate the actions of NMDA at it's receptor system on striatal neurons (see Kemp and Weiss, Soc. Neurosci. Abs., this volume). In conclusion, of the agents tested in this study, 56mM KCl is most effective in evoking the release of GABA from striatal neurons, while glycine release was most significantly increased by kainate. These data suggest that the modulatory actions of endogenous glycine at the NMDA receptor system on striatal neurons may be influenced by excitatory amino acid activation of the kainate receptor system.

Supported by the Medical Research Council of Canada.

461.11

MATURE HIPPOCAMPAL SLICES CULTURED 4 DAYS IN SYNTHETIC MEDIUM PLUS BSA, RELEASE D-^{3}H-ASPARTATE AND ^{14}C-GABA IN RESPONSE TO KAINATE(KA), QUISQUALATE(Q) OR 50mM K^+. N. Kleinberger-Doron* and M. Schramm. Dept. Biol. Chem. Herbew Univ. Jerusalem, Israel.

Slices from 45 day rats are sustained in a CSF-like, amino-acid medium. After 4 days slices show 90% of the uptake of the labeled amino acids, relative to fresh slices. 50mM K^+ releases D-^{3}H-aspartate and ^{14}C-GABA to the extent of 90% and 70% of that of fresh slices, respectively. In absence of added Ca^{2+} (10mM Mg^{2+}), release of both labeled amino acids is reduced by 68%.

Q and Ka, 60uM, induce in cultured slices a low, but significant, release of D-^{3}H-aspartate (1.2%-1.6% of total, after subtracting a basal release of 0.8%) and of ^{14}C-GABA (0.4%-0.7% after subtracting a basal release of 0.8%). Fresh slices demonstrate a similar release of ^{14}C-GABA, but rarely any of D-^{3}H-aspartate. A combined pulse of Ka+Q produces in the cultured slices an additive release of ^{14}C-GABA, but only half of the expected additive release of D-^{3}H-aspartate. Experiments with TTX and with Ca^{++} removal, suggest that Q may bring about inhibition of the Ka induced release. Our studies show that a mature CNS preparation can be maintained in vitro for days, retaining important functions of the tissue.

461.12

MODULATION OF ^{3}H-D-ASPARTATE RELEASE FROM THE RETINA DURING ONTOGENY. F. Somohano and A.M. López-Colomé. Instituto de Fisiología Celular, UNAM. Apdo. Postal 70-600. 04510 México, D.F. México.

Excitatory amino acids (EAA) participate as transmitters in the retina. We have previously demonstrated the presence of presynaptic receptors which modulate stimulated release of EAA. We have also demonstrated maturational changes in EAA synaptic receptors during ontogeny in the chick retina, which could be related to differentiation. Since the activity of these receptors depends on the availability of transmitter, which in turn is under presynaptic control, we studied the effect of EAA analogues on the K^+-stimulated release of ^{3}H-D-Aspartate from chick embryo retinas at days 7,8,11,14,18 and 21 of embryonic development (ED). Our results indicate the presence of K^+-stimulated release since day 7ED, however, the Ca^{++}-dependence of release increases gradually from 32% to 87% from ED7 to ED21. NMDA and KA were potent inhibitors of release, effect starting at day 14ED, while L-glutamate was less potent, showing effect from day 11ED. A ten fold increase in non-stimulated release of D-Asp was observed from ED 14 to 20, peaking at 18. Data suggest that while Ca^{++}-independent release of EAA could exert a trophic influence through the activation of postsynaptic receptors, once these structures acquire their mature characteristics, the amount of EAA present in the synaptic space, becomes controlled through presynaptic receptors.

461.13

MODULATION OF THE RELEASE OF ENDOGENOUS GLUTAMATE FROM RAT BRAIN SYNAPTOSOMES BY PUTATIVE PRESYNAPTIC RECEPTORS
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Rat brain synaptosomes were used as a model system for characterizing the modulation of the release of endogenous glutamic acid induced with high concentrations of K^+. L-2-amino-4-phosphono-butyric acid (L-AP4) inhibited the Ca^{2+} - dependent release of glutamate in a dose - dependent way, without affecting Ca^{2+} - independent or basal release. Kainic acid, N-methyl-D-aspartate (NMDA) and α-amino-3-hydroxy -5-methylisoxazole-4-propionate (AMPA) had weak or no effects on glutamate release. On the other hand, an inhibition was also found with L-AP7 and the L-enantiomer of the selective NMDA antagonist (E)-3-(2-carboxypiperazin-4-yl)-1-propenyl-1-phosphonic acid (CPP-ene, see Herrling et al., this volume), but not with D-CPP-ene. The non-NMDA-antagonist 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX) was able to reverse the inhibition of glutamate release seen with L-AP4. This action of L-AP4 was found in synaptosomes prepared from hippocampus or striatum, but not in cortical synaptosomes. It is concluded that L-AP4 inhibits the depolarization-induced exocytotic release of glutamate via presynaptic receptors which are not uniformly distributed throughout the brain and which are pharmacologically different from NMDA-, kainate- and quisqualate - subtypes of excitatory amino acid receptors.

461.14

MODULATION OF VIP-STIMULATED cAMP FORMATION REVEALS THREE CLASSES OF EXCITATORY AMINOACIDS (EAA) RESPONSES IN MOUSE CEREBRAL CORTICAL SLICES.
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Glutamate (GLU) and aspartate (ASP) potentiated in a concentration-dependent manner the effect of VIP on cAMP formation. In order to characterize the type of receptor involved, we have used three prototypical EAA receptor agonists. Kainate (KAI) mimicked the effect of GLU, NMDA was inactive and quisqualate displayed an inhibitory action. Furthermore, ibotenate (IBO) also potentiated the effect of VIP, while L-homocysteate (L-HCA) exhibited a stereospecific inhibitory action. IBO was 4 fold more potent and 2.5 times more effective than GLU (EC_{50} = 60 and 200 µM respectively). However, the effects of KAI and IBO were not additive, suggesting an activation of a common receptor. Thus, based on their effects on VIP-stimulated cAMP formation, EAA can be grouped in the three classes: those that potentiate the effect of VIP, such as GLU, ASP, KAI and IBO; (ii) those that inhibit the effect of VIP, such as L-HCA and quisqualate and (iii) those that are ineffective, such as NMDA and D-HCA. The effects of GLU or IBO were completely inhibited by L-phosphoserine and only partially by kynurenate. In a low chloride medium, or in the presence of TMB-8, an inhibitor of calcium release from internal stores, EAA did not potentiate the effect of VIP, thus stressing the importance of these ions for the transduction of the glutamatergic signal.

461.15

CYTOSOLIC CALCIUM RISE MEDIATED BY NON-NMDA RECEPTORS IN CULTURED RAT HIPPOCAMPAL NEURONS.
A. Ogura*, K. Akita* and Y. Kudo*. (Spon: S. T. Inouye). Mitsubishi Kasei Institute of Life Sciences, Tokyo 194, Japan.

It is postulated that the elevation of intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) in the postsynaptic cytoplasm plays an essential role in the modulation of synaptic transmission. From electrophysiological evidence, NMDA(N)-subspecies of excitatory amino acid receptor is regarded to be responsible for the $[Ca^{2+}]_i$ rise. Does it then mean that the synapses devoid of the N-subspecies modulate the transmission by Ca^{2+} independent mechanism? We suggest here not necessarily so by presenting evidence that non-N-subspecies are capable of raising the $[Ca^{2+}]_i$ as well.

Fluorometry with fura-2 showed that the $[Ca^{2+}]_i$ of cultured rat hippocampal neurons rose significantly upon exposure to kainate (K) or quisqualate (Q), even when voltage dependent Ca^{2+} channel was totally blocked by La^{3+} or ω-conotoxin/nitrendipine. Neither aminophosphonovalerate or Mg^{2+} suppressed the K- and Q-mediated responses. When external Ca^{2+} was replaced with Mn^{2+}, extinction of fluorescence was triggered by K and Q. Withdrawal of external Ca^{2+} annihilated and reduced the K- and Q-mediated responses, respectively. After excluding the possibilities of Ca^{2+} entry through voltage dependent Na^+ channel by tetrodotoxin and of Ca^{2+} accumulation due to retarded Na^+/Ca^{2+} antiporter by Na^+ depletion, we concluded that the K-subspecies induced Ca^{2+} entry and that the Q-subspecies produced both Ca^{2+} entry and Ca^{2+} release from internal storage.

461.16

GLUTAMATE STIMULATES RELEASE OF Ca FROM INTERNAL STORES IN ASTROCYTES.
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We report here that activation of glutamate receptors in astrocytes in the absence of external Ca produces an increase in intracellular Ca (Ca_i). Dissociated fetal rat spinal cord cultures (1-3 wk old) were used. Ca_i from the soma was measured with Fura-2 using the ratiometric (~2 ms/ratio) method. Astrocytes were identified by immuno staining for GFAP. Agonists were added in Ca-free solution (1mM EGTA,500 nM TTX) 3-5 min after prewash in the same.

Glutamate (>1 uM) and quisqualate (>0.1 uM) consistently caused a transient increase in Ca_i, with both the response amplitude and latency being graded functions of agonist concentration. Repeated stimulation at 4-7 min intervals produced similar responses (±10%). Within 1-2 sec of application, 100 uM glutamate or quisqualate produced 300 ± 100 nM increases in Ca_i which peaked within 1-2 sec. Recovery to resting Ca_i level was 60-80% complete within 2-3 sec. Some cells produced multiple transients which persisted even after withdrawal of the agonist. NMDA, kainate and aspartate did not produce any response, but 2-amino-4-phosphonobutyrate was as effective as quisqualate. We conclude that the quisqualate subtype of glutamate receptors mediates release of Ca from internal stores in astrocytes. Supported by NIH grant no. NS 27144 to ZA.

461.17

ANTIPROLIFERATIVE ACTION OF EXCITATORY AMINO ACIDS IN CULTURED ASTROCYTES.
F. Nicoletti*, F. Ingrao§*, G. Magrì§*, V. Bruno*, M.V. Catania*, P. Dell'Albani§*, D.F. Condorelli§* and R. Avola§* (SPON: B.L. Roth) Depts. of Pharmacology and §Biochemistry, University of Catania, Italy.

Quisqualate, ibotenate and glutamate stimulate inositol phospholipid hydrolysis and reduce [^{3}H]thymidine incorporation in cultured astrocytes, but not in C6-glioma cells or in slices from human glioma. The action of quisqualate is attenuated by L-AP4, but not by other receptor antagonists, including kynurenate or CNQX. The antiproliferative action of quisqualate requires a lag time of at least 4 hours and occurs during the transition between G_o and G_1 phase of the mitotic cycle in cells synchronized to proliferate. This suggests that inhibition of [^{3}H]thymidine incorporation by excitatory amino acids is consequent to the activation of gene expression and induction of specific proteins. Accordingly, quisqualate and ibotenate induce a rapid and transient increase in the expression of the early inducible gene, c-fos, in cultured astrocytes. These results support the view that activation of specific excitatory amino acid receptors coupled to inositol phospholipid hydrolysis contribute to regulate proliferation of astrocytes in culture.

461.18

FLUORESCENCE MEASUREMENT OF CHANGES IN INTRACELLULAR Ca INDUCED BY EXCITATORY AMINO ACIDS IN CULTURED ASTROCYTES.
A. Jensen* and S.Y.Chiu. Neuroscience Training Program & Dept. of Neurophysiology, University of Wisconsin, Madison, WI 53706.

Astrocytes vastly outnumber neurons in mammalian brain and are potential targets for neurotransmitters. Indeed, cultured astrocytes are known to express receptors for glutamate, which is possibly the most widely used excitatory neurotransmitter in the brain. Agonist induced changes in intracellular [Ca] are thought to be an important mediator of cell activity. We now report measurements of intracellular [Ca] in confluent, monolayer cultures of neonatal rat cortical astrocytes (>95% GFAP+) using the indicator dye fura-2. Bath applications of 100-500 µm glutamate (GLU), quisqualate (QA), kainate (KA) and NMDA induce a rise in [Ca]i with an order of potency of GLU ~ QA > KA >>> NMDA. Both GLU and QA responses consist of a rapid initial transient (200-500 nM change in [Ca]i) often followed by a damped oscillation; KA responses, in comparison, are slower in onset and more sustained. Removal of external Ca (2 mM EGTA) virtually abolished the KA response, but significant [Ca]i rises still are elicited by GLU (~30% of control) and QA (~100%). Prior treatment with TPA (100 nM), a potent protein kinase C activator, leads to an almost complete block of the GLU response, but only a ~50% and ~30% block of the QA and KA responses, respectively. These observations are consistent with the hypothesis that GLU and QA induce both mobilization of internal Ca stores and Ca influx. In contrast, KA primarily induces Ca influx.

Supported by NS-23375 (NIH), RG-1839 (National Multiple Sclerosis Society) and a PEW Scholar Award in Biomedical Sciences to S.Y.C.

461.19

GLUTAMATE INDUCES OSCILLATING INTRACELLULAR AND PROPAGATING INTERCELLULAR CALCIUM WAVES IN ASTROCYTES. S. M. Finkbeiner*, A. H. Cornell-Bell*, M. S. Cooper*, C. F. Stevens and S. J Smith. Molecular Neurobiology, Yale Univ. Sch. of Med., New Haven, CT 06510.

Intracellular Ca^{2+} changes were studied in astrocytes cultured from the CA1 region of the rat hippocampus using time-lapse video microscopy, the Ca^{2+} indicator Fluo-3 AM and FITC optics. Intracellular Ca^{2+} rapidly rises upon bath application of 1-1000uM glutamate in almost all astrocytes. After this initial rise three responses were seen. In one response, the cell Ca_i^{2+} oscillates with nearly constant amplitude and frequency (period = 15 ± 3 sec, n = 20) during the experiment (5-10 min). In another type, the cell begins to oscillate with constant frequency while the amplitude dampens to an elevated baseline. In the third type, cells do not oscillate. Confluent glia may also participate in expansive Ca^{2+} waves which propagate from cell to cell and radiate from initiation centers. In Ca^{2+}-free/3 mM EGTA Ringers oscillations are seen, though damped, suggesting they are partly due to intracellular Ca^{2+} release. Quisqualate (100uM) induces transients and oscillations similar to 100 uM glutamate while NMDA (100 uM) with glycine (10 uM) has no effect. Kainate (100uM) causes a step-like rise in Ca_i^{2+}. Neither the selective NMDA antagonist APV (1mM) nor the putative glutamate receptor antagonist APB (1 mM) could antagonise glutamate's effects. However, the selective non-NMDA antagonist, CNQX (10-100 uM), markedly attenuates the oscillations and waves. Blocking the sodium-dependent glutamate uptake system by choline substitution enhances the amplitude of glutamate-induced oscillations. The above responses cease quickly when the agonist is removed. These results suggest astrocytes possess receptors preferring kainate and quisqualate. The oscillations are consistent with models of quisqualate activated phosphoinositol turnover. These findings indicate that intercellular communication, possibly through gap junctions, can establish long-range Ca^{2+} signalling in glial networks.

461.20

SODIUM-DEPENDENT D-ASPARTATE BINDING IS NOT A MEASURE OF PRESYNAPTIC NEURONAL UPTAKE SITES IN AN AUTORADIOGRAPHIC ASSAY. J.T. Greenamyre, D.S. Higgins* and A.B. Young. Univ. of Mich., Ann Arbor, MI 48104.

Binding of D-[^{3}H]aspartate to rat brain was examined in an autoradiographic assay. Binding was dependent on the presence of sodium ions, but not chloride ions, and was optimal at 2°C. D-Aspartate binding reached equilibrium in 20 min and remained stable for 45 min. Dissociation was rapid with a $t_{1/2}$ of 56 sec, but was not as fast as anticipated, perhaps because of some sequestration of ligand. Binding in striatum had a K_D of 6.8 ± 1.2 μM and B_{Max} of 49.4 ± 8.6 pmol/mg protein. L-Glutamate, unlabeled D-aspartate, and D,L-threo-hydroxyaspartic acid, each competed for D-[^{3}H]aspartate binding with IC_{50}s of 7.02 ± 4.3 μM, 5.4 ± 1.5 μM, and 2.54 ± 1.03 μM, respectively. N-methyl-D-aspartate, quisqualate, and kainate had no affinity for this site. The regional distribution of binding did not conform to that of neuronal uptake sites described by others. Striatal D-aspartate binding was unaffected by unilateral decortication or striatal quinolinic acid lesions. NMDA, quisqualate, and kainate receptors were reduced by 80-90% by quinolinate lesions. The lesion studies strongly suggest that this site is not associated with either presynaptic glutamatergic afferents or intrinsic neurons of the striatum; it may be associated with glia.

461.21

LOCALIZATION OF THE CEREBELLAR KAINATE RECEPTORS ON BERGMANN GLIAL CELLS. N. Eshhar[1,2], J.D.B Roberts[*2], V.I. Teichberg[1] and P.Somogyi[2], Neurobiol. Dept.[1], Weizmann Inst. of Science, Rehovot 76100, Israel; MRC Anat. Neuropharm. Unit[2], Dept. Pharmacol., Oxford, U.K.

A high density of kainate receptors has been reported to be present in the cerebellar molecular layer of the goldfish (Eshhar et al. Brain Res. 476, 57-70, 1989) and the chick (Gregor et al. EMBO J. 7,2673-2678,1988). The precise cellular and subcellular distribution of these kainate receptors has now been studied by EM immunocytochemistry using the monoclonal antibody IX-50 directed against the purified kainate receptor protein. Both pre- and postembedding immunoperoxidase and immunogold methods were used. Immunoreactivity was found associated only with Bergmann glial cells. Intracellularly, immunoreactivity is observed in the endoplasmic reticulum, Golgi apparatus and lysosomes, representing putative sites of the synthesis, glycosylation and degradation of the kainate receptor. Extracellular immunoreactivity appears to be uniformly distributed along the Bergmann glial plasma membrane as confirmed by high resolution light microscopy. Bergmann glial processes form nests to the parallel fibre /Purkinje cell spine synaptic complexes. The presence of kainate receptors on Bergmann glia in close association with the spine synapses provides a basis for parallel fiber signalling to the Bergmann glia, presumably via glutamate.

EXCITATORY AMINO ACIDS: RECEPTORS VIII

462.1

QUANTITATIVE AUTORADIOGRAPHIC DETERMINATION OF THE DISTRIBUTION OF BINDING SITES FOR ^{3}H-MK-801 IN HUMAN HIPPOCAMPUS. **P. McGonigle, N. Lexow, M. A. Dichter and M. J. O'Connor. Departments of Pharmacology and Neurology, University of Pennsylvania School of Medicine and Department of Neurosurgery, Graduate Hospital, Philadelphia, Pennsylvania.**

The binding of ^{3}H-MK-801, a noncompetitive antagonist of the NMDA subtype of glutamate receptors, was measured in hippocampal tissue obtained from post-mortem specimens and patients undergoing temporal lobectomy to alleviate epileptic seizures. 20 μm thick sections of tissue were incubated in a 30 mM EPPS buffer solution containing various concentrations of ^{3}H-MK-801 for 2.5 hr at 22°C. Glycine (100 μM) and glutamate (100 μM) were included in the buffer and nonspecific binding was defined with 200 μM ketamine. Following a wash in ice-cold buffer, the sections were rapidly dried and apposed to LKB ultrofilm for 15 d. In post-mortem tissue, the highest densities of binding sites were observed in the CA_1 region, followed by the CA_2, CA_3, CA_4 regions and the dentate gyrus. These measurements were in good agreement with previous determinations of NMDA receptor density carried out with ^{3}H-glutamate. In temporal lobectomy tissue, the density of binding sites in the dentate gyrus was much higher than in post-mortem tissue. In some subjects, the relative distribution of binding sites was markedly different from the post-mortem samples, with the highest density of sites in the dentate gyrus and little or no binding detectable in the CA_1 and CA_2 regions. These results suggest that there are significant changes in the density and distribution of NMDA receptors as a consequence of epilepsy. (Supported by USPHS GM 34781 and the Pew Charitable Trusts)

462.2

CHARACTERIZATION AND DISTRIBUTION OF ^{3}H-MK-801 BINDING SITES IN THE RAT BRAIN. **S. Subramaniam and P. McGonigle. Department of Pharmacology, University of Pennsylvania School of Medicine, Philadelphia, PA 19104.**

Kinetics and optimal conditions for the binding of ^{3}H-MK-801, a non-competitive antagonist at the NMDA receptor, were determined using sections of mash prepared from rat brain. 20 μM thick sections were incubated at 22° C with ^{3}H-MK-801 (2-50 nM) in 30 mM EPPS containing 100 μM glutamate and 100 μM glycine. Non-specific binding was determined in the presence of 200 μM ketamine. Equilibrium was reached at 150 min. Sections were then rinsed in ice-cold buffer, wiped off and counted in scintillation fluid. Equilibrium and kinetic determinations of K_d were in good agreement. For densitometric analysis, sections were rapidly dried after the rinse and exposed to Ultrofilm for 15-21 d. Scatchard analysis of ^{3}H-MK-801 binding in the CA_1 region of the hippocampus yielded a Kd of 6.2 nM. The density of ^{3}H-MK-801 binding sites was determined in 34 regions at a concentration of 32 nM. These values corresponded to the density of NMDA receptors reported previously suggesting a 1:1 stoichiometric relationship for the two sites. The highest density of ^{3}H-MK-801 binding sites was in the CA_1 (stratum radiatum) (4213 fmol/mg). High densities were also found in the frontal cortical regions (3370 fmol/mg), primary olfactory cortex (3146 fmol/mg) and the olfactory tubercle (2415 fmol/mg). The density of ^{3}H-MK-801 binding sites in the hippocampus of rats kindled with pentylenetetrazole appeared to change, which is consistent with a role for the NMDA receptor in the kindling model of epilepsy. (Supported by USPHS GM 34781 and the Pew Charitable Trusts).

462.3

A UNIQUE GLUTAMATE BINDING SITE IN AN AUTORADIOGRAPHIC ASSAY. DS Higgins*JT Greenamyre,JJ Cha,JB Penney,AB Young. Department of Neurology, University of Michigan, Ann Arbor, MI 48104.

Glutamate receptor pharmacology identifies 3 postsynaptic receptor types: N-methyl-D-aspartate, quisqualate and kainate, each of which is labelled by (3H)glutamate. We describe a unique neuronal glutamate recognition site measured in the presence of saturating concentrations of NMDA, quisqualate and kainate in rat brain. In Tris buffer binding was enhanced by the presence of chloride and calcium ions and was maximal at 2° C. Glutamate bound rapidly, reaching equilibrium by 15 min and dissociated with a $t_{1/2}$ of 24 sec. Kinetic analysis yielded a K_D of 0.96± 0.09uM while scatchard analysis gave a K_D of 1.05± 0.25uM and a B_{max} of 2.22± 0.36pmol/mgprot, with a Hill coefficient of 0.90± 0.04. Binding was highest in outer cortical laminae, dentate gyrus and striatum. A wide variety of glutamate agonists and antagonists, as well as SITS and DIDS, did not alter binding. Binding in striatum was unaffected by ipsilateral decortication but was reduced by 65% in striatal quinolinate lesions. These results suggest that this is a unique postsynaptic neuronal site; it's function is not yet known. Supported by USPHS grants NS19613, AG06155 and NS07222.

462.4

IBOTENATE DISPLACES [^{3}H] GLUTAMATE FROM AN AMPA-INSENSITIVE QUISQUALATE BINDING SITE. J.J. Cha, J.B. Penney and A.B. Young. Neuroscience Prog. and Dept. Neurology, Univ. Michigan, Ann Arbor, MI 48104-1687

[RS]-α-Amino-3-hydroxy-5-methylisonazole-4-propionic acid (AMPA) is a selective glutamate agonist at the quisqualate class of excitatory amino acid receptor. However, AMPA displaces only a portion of quisqualate-sensitive [^{3}H] glutamate binding in rat brain. We investigated the properties of this "AMPA-insensitive, quisqualate sensitive [^{3}H] glutamate binding" (AiQsGB) in rat brain using a quantitative autoradiographic assay.

AiQsGB was measured in 50 mM Tris HCl buffer plus 2.5 mM $CaCl_2$ and 10 μM AMPA. Nonspecific binding was determined in the presence of 2.5 μM quisqualate. AiQsGB had a unique distribution within the rat brain (highest to lowest): cerebellar molecular layer > outer cortex > hippocampus > inner cortex > striatum > globus pallidus. AiQsGB was increased at 4° C vs. 37 ° C, distinguishing it from other quisqualate-sensitive glutamate binding processes. Similarly, the anion channel blocker SITS, which inhibits the chloride dependent uptake of [^{3}H] glutamate into membrane vesicles, was an ineffective blocker of AiQsGB with an IC_{50} > 100 μM. Other agents which were relatively ineffective at blocking AiQsGB (i.e. IC_{50} > 100 μM) were cystine, kynurenate, L-APB, BOAA, CNQX and DNQX. Effective displacers of AiQsGB were (with approximate IC_{50} values) quisqualate (10-20 nM), glutamate (200-300 nM), and ibotenate (2-3 μM).

AMPA-insensitive, quisqualate-sensitive [^{3}H] glutamate binding may represent binding to the novel type of quisqualate receptor believed to be linked to phosphoinositide metabolism.

Supported by USPHS grant NS 19613 and NIH NRSA 5T32.

462.5

THIOCYANATE INCREASES THE AFFINITY OF A LOW-AFFINITY BINDING SITE FOR AMPA. Z.R. Hollingsworth, J.J. Cha, J.B. Penney and A.B. Young. Neuroscience Prog. and Dept. Neurology, Univ. Michigan, Ann Arbor, MI 48104-1687.

We have investigated the pharmacological properties and regional distribution of binding of the glutamate receptor agonist [^{3}H](RS)-α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid ([^{3}H]AMPA) in rat brain using quantitative autoradiography.

[^{3}H]AMPA binding was highest in the hippocampus, cortex and cerebellum. Quisqualate and the quinoxalinedione compounds CNQX and DNQX were the most potent displacers of AMPA binding with IC_{50} values in the nanomolar range. Glutamate and BOAA also displaced AMPA binding with IC_{50} values in the low micromolar range. Ibotenate had no effect on AMPA binding. Regional analysis confirmed that AMPA binds to a subpopulation of quisqualate-sensitive glutamate binding sites.

Potassium thiocyanate (KSCN) increased [^{3}H]AMPA binding at all concentrations of KSCN tested (0 - 100 mM). In the presence of 100 mM KSCN, Scatchard analysis revealed two binding sites for AMPA. In the absence of KSCN, Scatchard analysis revealed an apparent single binding site. However, in saturation experiments using very high concentrations of [^{3}H]AMPA, a second binding site was detected in the absence of KSCN. The regional distributions of [^{3}H]AMPA binding in the absence of KSCN and in the presence of 100 mM KSCN were identical (r = .96). Scatchard analyses performed in the presence of intermediate concentrations of KSCN (1 and 10 mM) suggest that KSCN stimulated [^{3}H]AMPA binding by increasing the affinity of a low-affinity site. These data suggest that AMPA binding sites can exist in two conformations, and that the equilibrium between these two sites can be influenced by KSCN. Supported by USPHS grant NS 19613 and NIH NRSA 5T32.

462.6

REGULATION OF CEREBELLAR QUISQUALATE RECEPTORS IN PURKINJE CELL DEFICIENT AND GRANULOPRIVAL MICE. R.L. Makowiec*, J.J. Cha, J.B. Penney, and A.B. Young. Dept. Neurology, University of Michigan, Ann Arbor, MI 48109.

Quisqualate and AMPA ([RS]-α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid) act as potent agonists at a subset of excitatory amino acid receptors linked to ion channels (the quisqualate receptor). The regulation and properties of quisqualate-sensitive [^{3}H]-glutamate and [^{3}H]-AMPA binding were investigated in normal, Purkinje cell deficient (nervous nr/nr mutants) and granuloprival (methylazoxymethanol, MAM) mouse cerebella using a quantitative autoradiographic assay.

In the molecular layer of nervous mice lacking Purkinje cells, quisqualate-sensitive [^{3}H]-glutamate binding sites were reduced to 47% ($p < .003$) of control. [^{3}H]-AMPA binding sites were reduced to 25% ($p < .001$) of control. In cerebella of MAM treated mice adjacent to severe granule cell depletion, quisqualate-sensitive [^{3}H]-glutamate binding sites were increased to 140% ($p < .02$) of control, while [^{3}H]-AMPA binding sites were increased to 156% ($p < .009$) of control.

The results suggest that quisqualate-sensitive [^{3}H]glutamate and [^{3}H]AMPA binding sites are located primarily on Purkinje cells in cerebellum and that these sites upregulate after denervation.

Supported by USPHS grant NS 19613 and 5T32 GM07863.

462.7

Excitatory Amino Acid (EAA) Receptor Binding in Epileptic Human Hippocampi. D.A.Hosford, Z.Cao*, B.J.Crain, D.W.Bonhaus, A.H.Friedman, V.J.Nadler, & J.O.McNamara. Epilepsy Res. Lab, Duke Univ. & V.A. Med. Ctrs. Durham, NC, 27705.

Studies of the kindling model suggest that EAA-mediated neurotransmission may contribute to complex partial epilepsy in humans. We therefore used quantitative radiohistochemistry to measure the binding of ligands to NMDA receptors ([^{3}H]-glutamate displaced by NMDA), NMDA channels ([^{3}H]-TCP), and quisqualate receptors ([^{3}H]-AMPA) in sections of hippocampi surgically removed from patients with medically refractory complex partial seizures. Controls were hippocampi from autopsies of patients without epilepsy and without neurologic causes of death. Measurements were corrected for pathologic changes in neuronal density.

[^{3}H]-TCP and NMDA-displaceable [^{3}H]-glutamate binding were significantly decreased (33% and 61%, respectively, ($p < .05$) in area CA3 in specimens with mesial sclerosis ($n = 8$) even after correction for decreased neuronal density. In contrast, [^{3}H]-AMPA binding was increased by 100% ($p < .02$) in dentate gyrus stratum moleculare from these same specimens, but was not significantly changed in CA3 or CA1. The decreased [^{3}H]-TCP and NMDA-displaceable [^{3}H]-glutamate binding may reflect a down-regulation of NMDA receptor/channel function in surviving neurons. The increased [^{3}H]-AMPA binding in epileptic human hippocampi may indicate enhanced function of the same type of synapses that underlie longterm potentiation in animal models.

462.8

EFFECTS OF AGE ON GLUTAMATE DISPLACEABLE KAINIC ACID BINDING IN MOUSE BRAIN. J.C. MATTHEWS DEPT. OF PHARMACOLOGY AND RESEARCH INST. OF PHARMACEUTICAL SCIENCES, SCHOOL OF PHARMACY, UNIV. OF MISSISSIPPI, UNIVERSITY, MS 38677

Female mice at 2,5,10 and 20 months of age, produced at the University of Mississippi by brother-sister matings from C57Bl6Nnia stock, were used for analysis of the effects of age on brain kainic acid preferring glutamate receptors. Upon sacrifice brains were divided into cerebral cortex, remaining forebrain and hindbrain. Washed, frozen and thawed, whole particulate membranes were prepared. Replicate groups of 4 mice each at each age were employed for analysis of glutamate displaceable [^{3}H]-kainic acid binding with a centrifugation method. In the hindbrain, the affinity constant for glutamate displaceable kainic acid binding decreased as a function of increasing age while the number and density of binding sites remained constant. In the cerebral cortex the affinity constant was increased in the 20 month old animals relative to the younger age groups which were not different from one another. The number and density of binding sites in the cerebral cortex was constant over all age groups. In the forebrain the affinity and number of binding sites were unaffected by age. The cerebral cortex and hindbrain tissues did not change in weight over the age range tested, but the forebrain increased in weight with increasing age. Since the number of receptors in forebrain remained constant with age it can be concluded that the density of receptors in the forebrain decreased with age.

462.9

DECREASED NUMBER OF N-METHYL-D-ASPARTATE RECEPTOR-GATED ION CHANNELS IN HIPPOCAMPAL MEMBRANES OF AGED RATS. W.B. Perry*, D.W.Bonhaus, and J.O.McNamara (SPON:T. Slotkin). Duke Univ. and VA Medical Centers, Durham, NC 27705.

Aging is associated with a reduction in several forms of neuronal plasticity: older rats learn spatial tasks and kindle at a slower rate than young adults. Pharmacological studies suggest that activation of N-methyl-D-aspartate (NMDA) receptors is critical to these events. A change intrinsic to the NMDA receptor-gated ion channel may be responsible for the observed age-related deficits. To test this idea, we measured the binding of [^{3}H]-N-1-thienyl[cyclohexyl]piperidine (TCP) to the NMDA channel of hippocampal membranes from 3 and 24 month old Fisher-344 male rats. Equilibrium analysis of TCP binding isotherms (0.5-100nM TCP, 500 min) showed a decrease in maximum binding with no change in Kd.

Age (months)	Bmax (pmol/mg)	Kd (nM)
3	3.14 ± 0.37	19.9 ± 1.7
24	1.95 ± 0.17*	16.3 ± 1.9

Values are mean ± S.E.M. *Significantly different by Student's t-test, $p<0.02$, $n=4$.

This reduction in the number of NMDA channels supports the idea that a change intrinsic to this receptor/channel complex contributes to the decreased plasticity observed in the aged brain.

462.10

AGE-RELATED CHANGES OF AMINO ACID RECEPTORS IN THE RAT BRAIN STUDIED BY IN VITRO AUTORADIOGRAPHY. R. Miyoshi, S. Kito, N. Doudou* and S. Katayama*. Third Department of Internal Medicine, Hiroshima University School of Medicine, Hiroshima 734, Japan.

Glutamate appears to be a major excitatory transmitter in the mammalian central nervous system. Central excitatory amino acid receptors have been thought to be divided into three subclasses referred as N-methyl-D-aspartic acid (NMDA)-, quisqualic acid (QA)- and kainic acid-sensitive sites. Among these subtypes, NMDA sites are proposed to be involved in various central events such as neuronal plasticity, hypoxic neuronal death and long-term potentiation. In addition, strychinine-insensitive glycine receptors seem to be strongly related to NMDA receptors. In the present study, autoradiographic distributions of ^{3}H-CPP (a NMDA receptor antagonist), ^{3}H-AMPA (an agonist for QA receptors) and ^{3}H-glycine were investigated in the rat brain. Binding sites of each ligand whose Kd values were nanomolar-order were obtained by Triton X-100 treatment of tissue sections. High densities of each ligand were observed in the hippocampus, cerebral cortex, amygdala, olfactory tubercle and striatum. In addition, alterations of receptor distributions were also studied using the aged brain. Amino acid receptors in the hippocampus were decreased in parallel with aging. Hippocampal amino acid receptors may play an important role in brain aging.

462.11

ANTI-IDIOTYPIC ANTIBODIES AS INTERNAL IMAGES OF GLUTAMATE AND THEIR IMMUNOCYTOCHEMICAL APPLICATION IN THE RAT BRAIN. G. CAMPISTRON, P. DUBOURG* M. GEFFARD and A. CALAS*. Neuroimmunology, IBCN-CNRS. and *Neurobiology URA 339 CNRS, Bordeaux, FRANCE.

Idiotypic poly- and monoclonal anti-conjugated glutamate (Glu) antibodies or Ab1 allowed us to induce anti-idiotypic Glu antibodies or Ab2 in rabbits after alternative immunization. These latters were affinity chromatographed on rabbit and mouse non-immune immunoglobulins in order to remove anti-isotypic and anti-allotypic antibodies. Using ELISA tests, we performed competition experiments between Glu-conjugates coated on well-plates and purified Ab2 previously incubated with poly- and monoclonal Ab1. The displacement curves showed that Ab2 were internal images of conjugated Glu. To demonstrate the Ab1-Ab2 recognition specificity, we incubated the Ab2 Glu with other idiotypic antibodies directed against conjugated neurotransmitters (serotonin, dopamine, acetylcholine) and their respective conjugates and found no idiotypic binding. Considering this, we attempted to use the Ab2 for immunocytochemistry. Vibratome sections of paraformaldehyde-fixed rat brains were treated with 1/500 Ab2 subsequently revealed by PAP technique. In the hippocampus at the ultrastructural level, most of the immunoreactive structures were post-synaptic thickenings on dendritic or occasionnally somatic spines. Combined treatments with Ab2 and Glu-conjugates induced a sharp decrease of immunoreactivity. These data suggest that our anti-idiotypic Glu antibodies can specifically recognize Glu receptors and open new possibilities for their ultrastructural and biochemical characterization.

462.12

DIFFERENTIAL RESPONSE OF HIPPOCAMPAL EXCITATORY AMINO ACID RECEPTORS TO DEAFFERENTATION AND REINNERVATION. J. Ulas*, D.T. Monaghan[1] and C.W. Cotman (SPON: ENA) Depts. of Psychobiology and [1]Surgery, University of California, Irvine, CA 92717.

In vitro autoradiography was used to monitor the response of two subclasses of excitatory amino acid receptors, N-methyl-D-aspartate (NMDA) and quisqualate (QA), in the rat hippocampus after unilateral lesions of the entorhinal cortex (EC).

In response to EC lesions changes in NMDA and QA receptor binding levels were found not only in the outer two-thirds of the ipsilateral molecular layer (IML) but also in the inner IML indicating that the response of receptors is not restricted to directly denervated areas. An early decrease (5-20%) in the binding density of NMDA and QA receptors in the IML during the first week postlesion was followed by an increase in binding levels. Thirty to sixty days postlesion the binding levels of both receptors in the IML were higher than those in unoperated rats. The response of NMDA receptors (20-50% increase over control) was more pronounced than that of QA receptors. An increase in NMDA receptor binding levels (10-15% over control) was also found in the contralateral ML three to sixty days postlesion. No such changes were observed for QA receptors.

The data indicate that NMDA and QA receptors are differentially regulated in response to deafferentation. Comparison of these results to the time course of deafferentation and reinnervation suggests that the observed changes may be important components in the functional restoration of partially damaged circuitry.

462.13

EXCITATORY AMINO ACID RECEPTORS IN HUMAN CEREBRAL CORTEX: AUTORADIOGRAPHIC DISTRIBUTIONS OF [^{3}H]TCP, [^{3}H]GLYCINE, L-[^{3}H]GLUTAMATE, [^{3}H]AMPA, AND [^{3}H]KAINIC ACID BINDING SITES. K.L.R. Jansen,* R.L.M. Faull and M. Dragunow. Anatomy Dept., Univ. Auckland School of Medicine, Private Bag, Auckland, New Zealand.

The distributions of [^{3}H]1-(1-(2-thienyl)-cyclohexyl) piperidine ([^{3}H]TCP), [^{3}H]glycine, L-[^{3}H]glutamate, [^{3}H]-α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid ([^{3}H]AMPA) and [^{3}H]kainic acid ([^{3}H]KA) binding sites were studied in the human cerebral cortex. Under the appropriate conditions, [^{3}H]TCP, [^{3}H]glycine and L-[^{3}H]glutamate label sites on the N-methyl-D-aspartate (NMDA) receptor, while [^{3}H]AMPA labels the quisqualate and [^{3}H]KA the KA receptor. Labelling methods were as previously described (Jansen, K. et al., Brain Res. 482: 174, 1989). [^{3}H]TCP, [^{3}H]glycine and L-[^{3}H]glutamate sites had a congruent distribution, supporting data indicating that these ligands bind to different sites on the same complex. [^{3}H]AMPA sites had a similar pattern, except in the striate (primary visual) cortex, where the band of NMDA sites in lamina IVCβ was not matched by [^{3}H]AMPA. The [^{3}H]KA pattern was usually complementary to the other ligands (eg. in neocortex there was a band of high [^{3}H]KA density over laminae V and VI, while the other ligands had high densities over laminae I-III), except in the primary motor area where the highest level of [^{3}H]KA binding was in the outer layer, supporting data from non-glutamatergic receptor studies suggesting a unique chemoarchitecture for this area (Lidow, M. et al., Soc. Neurosci. Abst. 14: 170, 1988). It thus appears that excitatory amino acid neurotransmission in the human brain, as in the rat, is mediated by anatomically distinct subsystems. Supported by NZ Medical Research Council.

463.1

INHIBITION OF GLUTAMATE-INDUCED FIRING IN HIPPOCAMPAL SLICES BY LOW GLUCOSE OR BY AMMONIUM IONS. Fan Ping* and J.C.Szerb. Dept.of Physiol.& Biophys.,Dalhousie Univ., Halifax N.S. B3H 4H7,Canada.

Synaptic transmission in hippocampal slices is depressed in low glucose medium (Cox & Bachelard, Brain Res.239:527,1982) or by NH_4^+ (Theoret et al. Neurosci. 14:798,1985). Whether this depression is pre- or postsynaptic is controversial. To test for a post-synaptic blockade, firing of units in the pyramidal layer in CA1 area induced by 100 msec pulses of electro-phoretic glutamate was tested. Lowering glucose from 5 to 0.2 mM reversibly inhibited glutamate induced firing by about 70%, but 2 or 5 mM NH_4Cl reduced it by only about 20%. If however, 25 μM bicuculline was present, which increased the response of neurons to glutamate, the effectiveness of NH_4Cl more than doubled. These observations agree with findings that neither low glucose (Szerb & O'Regan,Synapse, 1:265,1987), nor 5 mM NH_4Cl (Butterworth et al.Hepatic Encephalopathy,Humana Press, in press) inhibit stimulation-induced release of excitatory amino acids from hippocampal slices and suggest that synaptic transmission is blocked at a postsynaptic site. To reveal the blockade by NH_4^+ of glutamate-induced excitation, GABA-ergic inhibition has to be reduced, since, by interfering with the Cl^- pump, NH_4^+ also decreases this inhibition (Raabe, Brain Res. 210:311,1981). (Supported by the MRC of Canada.)

463.2

INHIBITION OF QUISQUALATE (QUIS)- AND IBOTENATE (IBO)-STIMULATED PHOSPHOINOSITIDE HYDROLYSIS IN THE NEONATAL RAT HIPPOCAMPUS BY 2-AMINO-3-PHOSPHONOPROPIONATE (D,L-AP3). D. D. Schoepp and B.G. Johnson*, Lilly Research Laboratories, Eli Lilly and Company, Indianapolis, IN 46285.

The pharmacological specificity of excitatory amino acid receptor coupling to brain phosphoinositide hydrolysis was characterized in hippocampal slices from 7 day old rats. Tissue slices were prelabeled with ^{3}H-inositol, washed with buffer containing 10mM LiCl, then incubated with agonists for 60 min. Antagonists were preincubated 20 min prior to the agonist incubation. Maximally effective concentrations of IBO (10^{-4}M) or QUIS (10^{-4}M) increased the formation of ^{3}H-inositol monophosphate (^{3}H-IP) by 11 to 12 fold over the basal values. L-Glutamate or L-aspartate enhanced ^{3}H-IP about 6-fold at 10^{-3}M. QUIS was the most potent agonist (EC50% 1.9μM), followed by IBO (EC50% 27μM), then L-glutamate (EC50% 458μM). AMPA or kainate increased ^{3}H-IP by 2-4 fold at 10^{-3}M. D,L-AP3 (10^{-3}M) completely inhibited IBO and QUIS stimulations, partially inhibited L-glutamate stimulation, but had no effect on AMPA-, kainate-, or carbachol-induced ^{3}H-IP. The ionotropic QUIS receptor antagonist DNQX (10^{-4}M) did not inhibit stimulations by QUIS or IBO. These studies show that excitatory amino acids stimulate phosphoinositide hydrolysis in the neonatal rat hippocampus by activating metabotropic QUIS receptors that can be selectively inhibited by D,L-AP3.

463.3

SR-95531 BLOCKS SELECTIVE UPREGULATION OF THE QUISQUALATE MEDIATED PHOSPHOINOSITIDE (PI) HYDROLYSIS IN CULTURED CEREBELLAR GRANULE CELLS INDUCED BY 7-DAY γ-AMINOBUTYRIC ACID (GABA) PRETREATMENT. Onnfoh Yu* and De-Maw Chuang, LPP, NIMH, St. Elizabeths Hosp., Washington D.C. 20032. (SPONSOR: An-Zhong Zhang)

Cultured cerebellar cells use glutamate as their neurotransmitter and express three l-glutamate receptor subtypes, namely; kainate, N-methyl-D-aspartate, and quisqualate receptors that are linked to PI hydrolysis by phospholipase C. Following 7 days of 50 μM GABA pretreatment, it was previously shown that the l-glutamate receptor subtype, quisqualate receptor was selectively upregulated. We examined the effect of SR-95531, a $GABA_A$-receptor antagonist and (±) baclofen, a $GABA_B$-receptor agonist to determine the type of GABA receptor involved in the upregulation of quisqualate receptor by 7-day GABA pretreatment. 7-day GABA pretreatment increased 100 μM quisqualate mediated PI hydrolysis (1.64 ± 0.31 fold of control, N = 5). SR-95531 produced a dose-dependent reduction of this GABA effect when added into the culture medium with GABA (SR-95531; 1 μM: 1.34 ± 0.16; 10 μM: 1.15 ± 0.20; 100 μM: 1.19 ± 0.09 fold of control; N = 5). In contrast, 7-day pretreatment of granule cells with 100 μM (±) baclofen did not significantly enhanced the PI hydrolysis induced by the excitatory amino acids. It is therefore concluded that $GABA_A$ receptor activation is involved in the upregulation of quisqualate receptor mediated PI hydrolysis by 7-day GABA pretreatment since $GABA_A$ receptor antagonist was able to block this effect and $GABA_B$ receptor agonist was not able to mimic the effect of GABA. This work was done while O.Y. was a Nat. Res. Council/NAS resident research associate at NIMH.

463.4

MECHANISMS OF ANTICONVULSANT ACTION OF CENTRALLY-ACTING ANTITUSSIVES IN HIPPOCAMPAL SLICES. J.P. Apland and D.J. Braitman, Neurotox Br, U S A Med Res Inst Chem Def, APG, MD 21010

Carbetapentane(CB), caramiphen(CM) and dextromethorphan(DM) are centrally-acting antitussives which block epileptiform bursting induced by Mg^{++}-free medium in hippocampal slices (Braitman and Apland, 1988). CM has cholinolytic activity (Kraatz et al., 1949), which might be considered to be the basis for its anticonvulsant properties. We present evidence here that the anticonvulsant activity of these antitussives is not based on their cholinolytic properties, but upon a mechanism common to all three. Extracellular recordings were obtained from area CA1 of guinea pig hippocampal slices in artificial cerebrospinal fluid (ACSF). Evoked responses were elicited by stimulation of the Schaffer collaterals. Atropine (5-300 uM) did not block spontaneous epileptiform bursting induced by Mg^{++}-free ACSF without also blocking evoked responses at the highest concentrations, whereas CM (30 uM) was effective. Pretreatment with DM (30-100 uM) blocked epileptiform afterdischarges following evoked responses in both Mg^{++}-free ACSF and NMDA-containing ACSF. In contrast, pretreatment with CB or CM (30-100 uM) prevented afterdischarges in Mg^{++}-free ACSF, but completely depressed evoked responses in ACSF containing NMDA without blocking NMDA-induced afterdischarges. This evidence suggests that neither cholinolytic properties nor NMDA antagonism are necessary for the anticonvulsant activity of these antitussives.

463.5

CHARACTERIZATION OF THE BINDING OF [^{3}H]CGP 39653 TO THE N-METHYL-D-ASPARTATE (NMDA) RECEPTOR. Matthew A. Sills, E. Jay Wilusz*, Christof Angst*, Derek E. Brundish*[1] and Michael Williams. Research Department, CIBA-GEIGY Corporation, Summit, NJ 07901 and CIBA-GEIGY Ltd, Horsham, England[1].

During the past several years, the NMDA antagonist radioligands [^{3}H]CPP (3-(±)-2-carboxypiperazin-4-yl)propyl-1-phosphonic acid) and [^{3}H]CGS 19755 (cis -4-phosphonomethyl-2-piperidine carboxylic acid) have gained utility as tools to measure binding to the NMDA receptor. CGP 39653 (E-2-amino-4-phosphonomethyl-3-heptenoic acid) was found to inhibit the binding of [^{3}H]CPP to the NMDA receptor with an IC_{50} value of 5.0 nM. The present study examined the characteristics of [^{3}H]CGP 39653 binding to rat forebrain membranes in a filtration assay. Specific binding of [^{3}H]CGP 39653 was saturable, reversible and reached steady state within 60 min and represented 70 - 80 % of total binding. Computer analysis of binding data from saturation experiments revealed a Kd value of 6.2 ± 0.6 nM and a Bmax value of 970 ± 222 fmol/mg protein. When a series of NMDA agonists and antagonists competed for the binding of 2.0 nM [^{3}H]CGP 39653, the following order of potency was generated (Ki) : CGS 19755 (43 nM) = L-glutamate (45 nM) > CPP (106 nM) > D-aspartate (460 nM) > NMDA (2700 nM). Quisqualate and kainate inhibited less than 65 % specific binding at 10 μM,generating steep inhibition curves with Hill coefficients near unity. In contrast, glycine produced a biphasic inhibition curve. Computer analysis indicated that 37 ± 1 % of the binding was inhibited with an IC_{50} value of 536 ± 98 nM, whereas 63 ± 1 % of the binding was inhibited with an IC_{50} value of 868 ± 103 μM. These results indicate that [^{3}H]CGP 39653 is a selective, high affinity radioligand for the NMDA receptor.

463.6

RECEPTOR-LIGAND INTERACTIONS OF THE NMDA-TYPE GLUTAMATE RECEPTOR AS STUDIED USING DIFFERENT N-(1-[2-thienyl] cyclohexyl)piperidine (TCP) DERIVATIVES. S.O. Casalotti*, W. Tuekmantel*, A. Fauq*, A.P. Kozikowski, and K.E. Krueger. FGIN, Georgetown Univ. Med. Sch., Washington, D.C. 20007 and Dept. of Chemistry, Univ. of Pittsburgh, Pittsburgh, PA 15260.

Different derivatives of TCP modified at the region corresponding to the piperidine substituent were synthesized and tested for their abilities to compete against specific [^{3}H]MK-801 binding to bovine cortical synaptosomal plasma membranes. Phencyclidine (PCP) and TCP inhibited [^{3}H]MK-801 binding with an IC_{50} of 150 nM and 20 nM, respectively. Substitution of the TCP piperidine ring with different N-alkyl substituents from one to five carbon atoms in length exhibited great variation in the ability to inhibit [^{3}H]MK-801 binding with the N-ethyl derivative being most potent. Additional compounds with much longer N-substituents demonstrate affinities comparable to that of the N-methyl compound. Introduction of polar groups on these N-substituted alkyl chains greatly reduces the apparent affinity for this receptor. These studies provide more extensive detail in understanding the structural features of the MK-801-TCP binding site and should be helpful in attempting to design affinity ligands for this site.

463.7

CORTICAL METABOLIC ACTIVITY IS DIFFERENTIALLY ALTERED BY COMPETITIVE (AP7) AND NONCOMPETITIVE (MK-801) NMDA ANTAGONISTS. S.J. Lee*, D.W. Clow, J.E. Margulies, D. Yoshishige* and R.P. Hammer, Jr. (SPON: D.H. Crowell). Dept. Anatomy & Reprod. Biol., Univ. Hawaii Sch. Med., Honolulu, HI 96822.

Noncompetitive NMDA antagonist compounds, such as ketamine, are known to preferentially decrease glucose utilization in primary sensory cortices and to shift glucose labeling in terminal afferent fields from layer IV to layer Va (Hammer and Henkenham, *J. Comp. Neurol.*, 220: 396). Selective activation of limbic cortical areas also occurs. We compared the pattern and density of cortical activity following various doses of DL-amino-7-phosphonoheptanoate (AP7), MK-801 or saline vehicle using the quantitative [^{14}C] 2-deoxyglucose (2DG) procedure.

Arterial and venous cannulae were implanted using halothane anesthesia in male, Sprague-Dawley rats. Four hours later, MK-801 (0.1 or 1.0 mg/kg) or AP7 (1.5 or 4.0 mmol/kg) were administered intravenously, and 2DG was given 10 min thereafter. Computer-assisted analyses of autoradiographs and the brain sections which produced them yielded lCGU values in defined laminae of various cortical regions. Histologic analysis allowed precise differentiation of primary somatosensory (S_I), visual (V_I), hippocampal and other cortical regions.

MK-801 selectively decreased lCGU in neocortex and increased lCGU in limbic regions. At the lower dose, the drug decreased lCGU in S_I, V_I and auditory cortex, but not in S_{II} or V_{II}, producing an apparent cortical columnar pattern. The effect was more pronounced in layer IV than Va, resulting in a relative activity shift to layer Va. In contrast, AP7 produced a generalized, dose-dependent reduction of lCGU in all layers of neocortical and limbic regions, with some sparing of motor and entorhinal cortex. Thus, the pattern of effect of these drugs in cortex is quite different, particularly in limbic and primary sensory cortex, suggesting differential mechanisms of action. (Supported by USPHS Award DA04081; AP7 was generously provided by J.B. Monahan at G.D. Searle & Co.)

463.8

DISCRIMINATIVE STIMULUS PROPERTIES OF THE NONCOMPETITIVE NMDA ANTAGONIST MK-801 IN THE RAT.
J. De Vry* and J. Traber*. (SPON: F.K. PIERAU), Department of Neurobiology, Troponwerke, Berliner Strasse 156, D-5000 Köln 80, F.R.G.

In order to characterize the discriminative stimulus properties of the noncompetitive NMDA antagonist MK-801 [(+)-5-methyl-10,11-dihydro-5H-dibenzo[a,d]cyclohepten-5,10-imine maleate], rats were trained to discriminate 0.1 mg/kg MK-801 (i.p.) from saline in a FR-10 food-reinforced two-lever procedure. Dose-dependent generalization was obtained with MK-801 (ED50 in mg/kg: 0.03), and with three other noncompetitive NMDA antagonists [phencyclidine (1.4), ketamine (5.4) and (+)-N-allylnormetazocine (12.9)]; but not with the competitive NMDA antagonist 3-(2-carboxypiperazin-4-yl) propyl-1-phosphonic acid (CCP). Partial generalization was obtained with diazepam and ipsapirone; possibly indicating a minor anxiolytic component of the MK-801 cue. No generalization was obtained with imipramine, apomorphine, (+)-3-PPP [(+)-3-(3-hydroxyphenyl)-N-(1-propyl)piperidine] and haloperidol. In antagonism tests, NMDA pretreatment failed to affect the MK-801 cue. Pretreatment with (+)-3-PPP or haloperidol partially antagonized the MK-801 cue, but this occurred at behaviorally disruptive doses. The results suggest that MK-801 shares the psychotomimetic properties of the dissociative anesthetics and that the behavioral effects of competitive and noncompetitive NMDA antagonists are dissimilar.

463.9

ACTIONS OF (-)SCOPOLAMINE AND ATROPINE ON NMDA RECEPTORS: IMPLICATIONS ON MEMORY AND LEARNING. I.N. Cestari[1]*, Y. Aracava[1,2]* and E.X. Albuquerque[1,2]. (SPON: M.P. Blaustein) [1]Lab. Mol. Pharmacol. II, UFRJ, Rio de Janeiro, Brazil & [2]Dept. Pharmacol., Univ. Md. Sch. Med., Baltimore, MD 21201.

(-)Scopolamine, an antimuscarinic agent, promotes amnesia and learning impairment at therapeutic doses. This effect was not reported for atropine and pirenzepine. In the hippocampus, the facilitation of NMDA-mediated synaptic transmission has been implicated in the acquisition of memory and learning processes which can be altered by many NMDA channel blockers. Therefore, the actions of (-)scopolamine and atropine on the kinetics of NMDA-activated currents were investigated in outside-out patches from cultured rat hippocampal neurons. In Mg^{2+}-free solution, NMDA (5-10 μM) activated bursts that showed increased flickering with hyperpolarization and two populations of closed times. At holding potentials between -75 and -120 mV, (-)scopolamine (5-100 μM) blocked the NMDA channels and reduced the frequency of bursts. The number of openings per burst increased with concentration and decreased with hyperpolarization. Mean open times was shortened while both populations of intraburst closures were prolonged with increased (-)scopolamine concentration. Comparatively, atropine (10-100 μM) produced lesser reduction of both frequency of openings and mean open time such that only at concentrations >50 μM could significant alteration be discerned. These results suggest that (-)scopolamine is the more effective of these drugs as a blocker of channels activated by NMDA. (Support: FINEP & CNPq, Brazil; US Army Med. Res. & Devel. Comm. Contract DAMD17-88-C-8119)

463.10

TACRINE AND AMINACRINE BLOCK CHANNELS GATED BY NMDA IN CULTURED HIPPOCAMPAL NEURONS. A.C.S. Costa* and E.X. Albuquerque. (Spon: A. Eldefrawi) Lab. Mol. Pharmacol. II, Fed. Univ. Rio de Janeiro, Brazil; Dept. Pharmacol. Exp. Ther., Univ. Maryland Sch. Med., Baltimore, MD 21201.

1,2,3,4-Tetrahydro-9-aminoacridine (tacrine) is an anticholinesterase agent which has been shown to be useful for long-term treatment of patients with Alzheimer's disease. This agent also has noncompetitive effects on peripheral nicotinic acetylcholine receptor (AChR)(see R.A.M. Reis et al., this meeting). Since many noncompetitive blockers of the AChR also have actions on the NMDA receptor, we decided to study the actions of both tacrine and the chemically related anti-infective agent 9-amino-acridine (aminacrine) on single channel currents (patch clamped in the outside-out configuration) mostly recorded at -85 mV from cultured hippocampal cells 8-21 days old. Tacrine and aminacrine significantly reduced the channel open time in a concentration-dependent fashion (10-50 μM range). Channel openings produced by NMDA (5-15 μM) occur in bursts, which seem to increase in length as we increased the concentration of either agent. We also observed a reduction in frequency of openings and single channel conductance at concentrations greater than 10 μM; however, at this point further analysis is needed to establish whether or not the latter effects represent a real ion channel blockade. The blocking effect of tacrine on NMDA-gated channels could be responsible for its protection against NMDA cytotoxicity and may represent an alternative mechanism of action for this and some related compounds on Alzheimer's disease. (Support: CAPES Proc. 4987/88-2; US Army Med. Res.& Devel. Comm. Contr. DAMD17-88-C-8119).

463.11

CPP AND PCP PRODUCE AMNESIA OF A PASSIVE AVOIDANCE RESPONSE IN RATS. V.J. DeNoble, K.W. Jones*, C.L. Schaeffer* and L.M. Bauerle*. E. I. du Pont de Nemours & Co., Med. Prod. Dept., Exp. Sta., P.O. Box 80400, Wilmington, DE 19880.

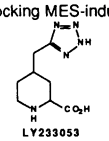
There are interactions between PCP and NMDA. PCP selectively antagonizes NMDA-induced neuronal excitation and EEA antagonists produce PCP-like behavioral effects. The NMDA antagonist CPP shares several characteristics with benzodiazepines (BZD). Since the amnestic effects of BZD have been established and are blocked by the BZD antagonist RO15-1788, the purpose of the present study was to first, directly compare the amnestic effects of CPP and PCP in a passive avoidance (PA) test; and second, determine if RO15-1788 would block the amnestic effects of CPP or PCP.

Pretraining administration with CPP (2.0-10.0 mg/kg sc) significantly decreased retention latencies 24 hrs after training. Similar effects were found with PCP at doses ranging from 0.3 to 1.7 mg/kg sc and diazepam at doses between 5.0 to 18.0 mg/kg sc. In contrast, pretraining administration of RO15-1788 at 0.1 to 15 mg/kg sc did not alter retention latencies. Co-administration of RO15-1788 (0.01 to 15 mg/kg sc) with CPP (6.0 mg/kg sc) or PCP (1.0 mg/kg sc) failed to block the amnesia. However, when RO15-1788 was co-administered with diazepam (9.0 mg/kg sc) a dose related antagonism of diazepam induced amnesia was found. These results suggest that the behavioral actions of CPP and PCP on PA retention are not mediated via the benzodiazepine receptor complex.

463.12

BEHAVIORAL PHARMACOLOGY AND DURATION OF ACTION OF LY233053: A STRUCTURALLY NOVEL NMDA RECEPTOR ANTAGONIST. J.D. Leander*, P.L. Ornstein, D.D. Schoepp, and C.R. Salhoff* (SPON: D. Goldstein). Lilly Research Laboratories, Eli Lilly and Company, Indianapolis, IN 46285

We report here the behavioral pharmacology profile, in mice, rats and pigeons, of the novel, selective and potent competitive NMDA receptor antagonist, LY233053 (cis-(±)-4-((2H-tetraazol-5-yl)methyl)piperidine-2-carboxylic acid). This compound was studied in a variety of *in vivo* assays. In mice, LY233053 protected against maximal electroshock-induced (MES) seizures and NMDA-induced lethality at lower minimal doses (10 mg/kg) than the minimal dose (40 mg/kg) which impaired horizontal screen performance. In rats, the ED_{50} against MES seizures was 3.5 mg/kg, i.p. In pigeons, LY233053 antagonized (ED_{50} = 1.3 mg/kg, i.m.) the complete behavioral suppressant effects of 10 mg/kg of NMDA. The dose of LY233053 estimated to shift the NMDA dose-response curve two-fold was 2.3 mg/kg. A dose of 40 mg/kg, i.m., did not produce the phencyclidine-like catalepsy described in pigeons. LY233053 blocked NMDA-induced convulsions in neonatal rats with a duration of action of 4 hours. This compared to a >12-hour duration of action for CGS-19755. The same relative duration of action was observed for LY233053 and CGS-19755 in blocking MES-induced convulsions in rats.



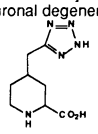
LY233053

LY 233053 demonstrates a short duration of action when compared to phosphonate substituted amino acids, good *in vivo* activity, and may be therapeutically advantageous in the treatment of certain excitatory amino acid related disorders.

463.13

IN VITRO AND *IN VIVO* CHARACTERIZATION OF LY233053: A STRUCTURALLY NOVEL COMPETITIVE NMDA RECEPTOR ANTAGONIST. P.L. Ornstein, D.D. Schoepp, J.D. Leander*, D.T. Wong, D. Lodge, C.R. Salhoff*, L.G. Mendelsohn, and N.R. Mason. Lilly Research Laboratories, Eli Lilly and Company, Indianapolis, IN 46285 and Royal Veterinary College, London, NW10TU, UK.

This study reports the activity of a structrually novel excitatory amino acid receptor antagonist, LY233053 (cis-(±)-4-((2H-tetraazol-5-yl)methyl)piperidine-2-carboxylic acid), the first tetrazole-containing competitve NMDA antagonist. LY233053 potently inhibited NMDA receptor binding to rat brain membranes as shown by the *in vitro* displacement of ^{3}H-CGS19755 (IC_{50} = 107 nM), ^{3}H-CPP (IC_{50} = 164 nM), and ^{3}H-MK801 (IC_{50} = 1103 nM). No appreciable affinity in ^{3}H-AMPA or ^{3}H-kainate binding assays was observed (IC_{50}s > 10,000 nM). NMDA receptor antagonist activity was further demonstrated by selective inhibition of NMDA-induced depolarization in cortical wedges (IC_{50} = 6.4 μM versus 40 μM NMDA). The CNS effects of NMDA were also antagonized following *in vivo* systemic administration of LY233053. LY233053 prevented NMDA-induced lethality in mice (MED [minimum effective dose] = 10mg/kg, i.p.) and NMDA-induced rat striatal cholinergic neuronal degeneration (MED = 100 mg/kg, i.p.).


LY233053

Thus, LY233053 is a potent, selective, and competitive antagonist of NMDA excitatory amino acid receptors *in vitro* and *in vivo*. The antagonist activity of this compound following systemic administration demonstrates potential therapeutic value in conditions of neuronal cell loss due to NMDA receptor excitotoxicity.

463.14

NOVEL PEPTIDE NMDA ANTAGONISTS FROM THE VENOM OF CONUS

J. Haack, E. Mena+, T. N. Parks**, J. Rivier#, L. Cruz and B. Olivera. Dept. of Biology, Univ. of Utah and **Natural Product Sciences, Salt Lake City, Utah, +Pfizer Central Research, Groton, CT. and #The Salk Institute, La Jolla, CA.

Two homologous peptides, conantokin-G (formerly called conotoxin GV) and conantokin-T have been isolated from the venom of the fish-hunting cone snails Conus geographus and Conus tulipa respectively. Both peptides elicit a sleep-like state in animals under two weeks of age and a hyperactive state in older mice. These peptides contain 4-5 residues of the unusual amino acid, γ-carboxyglutamate (Gla) in homologous positions. The Gla residues are required for expression of the profound behavioral effects of these peptides. We examined the ability of these peptides to affect CNS systems which could potentially mediate these behavioral effects. Our results show that these peptides represent a novel, potent class of peptide NMDA antagonists. Conantokin-G blocked the NMDA-induced (100 μM) elevation of cGMP in the rat cerebellum in vitro with an IC50 of 32 nM but had no effect on kainic acid induced (100 μM) elevations at 1 μM. Comparison of this activity with other NMDA antagonists in this assay shows that this peptide is approximately 2 and 200-fold more potent than MK-801 and AP7, respectively. Both peptides were able to attenuate the NMDA mediated influx of calcium in cultured cerebellar granular cells as monitored by fura-2-fluorescence. Conantokin-G also inhibited [^{3}H]L-Glu binding to synaptic junctions (IC50 of 940 nM), but had no effect on [^{3}H]TCP binding (IC50>>1 μM). Conantokin-G enhanced [^{3}H]Gly binding to rat forebrain membranes similarly to the polyamine compounds (e.g., ARG636) isolated from several species of spiders (see E. Mena et al. and M. Gullak et al., this meeting). Conantokin-G, however, did not block the binding of [^{125}I]Arg636 to rat brain membranes. The conantokins are the first functional peptide antagonists of NMDA to be reported. The mechanism by which they block the activation of NMDA receptors is currently under investigation.

463.15

ASCORBIC ACID AND GLUTATHIONE ARE ANTAGONISTS OF THE NMDA RECEPTOR. M.D. Majewska, J.M.H. ffrench-Mullen and E.D. London, Neuropharmacology Lab., Addiction Res. Ctr., NIDA, Baltimore, MD 21224 and Neurophysiology Lab., NINCDS, Bethesda, MD.

Ascorbic acid (AA) and glutathione (GSH) exist in the CNS at high micromolar to millimolar concentrations (Schenk, J.O., et al., Brain Res. 253:353, 1982; Slivka, A., et al., Brain Res. 409:275, 1987). They are localized mostly in glia, nerve terminals and the choroid plexus, and are released into CSF during neuronal activity. We examined the interaction of AA and GSH with NMDA receptors in the rat brain. At physiological concentrations (μM), AA and GSH inhibited binding of [^{3}H]glutamate and reduced the binding affinity of N-[1-(2-thienyl)cyclohexyl]piperidine ([^{3}H]TCP), a steric blocker of the NMDA-gated cationic channel, to brain membranes. These effects resembled those of competitive NMDA antagonists. In electrophysiological recordings, AA and GSH reversibly inhibited NMDA-gated inward currents in isolated hippocampal neurons. Our data suggest that AA and GSH act in the CNS as endogenous antagonists of NMDA receptors. As hyperactivity of NMDA receptors is linked to neuronal damage associated with ischemia, edema, trauma and some neurodegenerative diseases, these data imply that AA and GSH may function as neuroprotective agents in the CNS. Also, since the plasma and CSF levels of AA and GSH increase after ACTH stimulation (Briggs, F.N. and Toepel, W., Endocrinol. 62:24, 1958; Lahiri, S. and Lloyd, B., Biochem. J. 84:478, 1962), these agents may act as stress hormones.

463.16

N-(PHOSPHONOALKYL AND -ARYL SUBSTITUTED)-α-AMINO ACIDS AS COMPETITIVE INHIBITORS OF THE NMDA RECEPTOR. C.F. Bigge,* G. Johnson,* F.W. Marcoux, A.W. Probert, L.L. Coughenour,* L.J. Brance* (SPON: F. Hershenson). Parke-Davis Pharmaceutical Research Division, Warner-Lambert Company, 2800 Plymouth Road, Ann Arbor, MI 48103.

Two novel series of N-substituted amino acids with terminal phosphonic acid groups were prepared as potential competitive N-methyl-D-aspartate (NMDA) antagonists to examine the steric and entropic requirements for retention of affinity. An assay using crude rat brain synaptic membranes and [3H]-4-(3-phosphonopropyl)-2-piperazinecarboxylic acid ([3H]-CPP) as ligand was used to determine NMDA receptor affinity. Antagonist action was confirmed by their ability to inhibit glutamate-induced accumulation of [45Ca++] in cultured rat cortical neurons. Compounds found to have significant receptor affinity in the [3H]-CPP NMDA receptor assay were N-(4-phosphono-2-butenyl)glycine (IC50=1.2μM), N-[[3-(phosphonomethyl)phenyl]-methyl]glycine (IC50=2.24μM), N-(4-phosphono-3-butenyl) glycine (IC50=2.45μM) and N-[[4-(phosphonomethyl)phenyl]-methyl]glycine (IC50=5.2μM). Antagonist activity in the [45Ca++] influx assay in cell culture was well correlated to their receptor affinity. The compounds described support a folded conformation for antagonist binding at the receptor. Entropy appears to be the most important factor responsible for high affinity. Each of the compounds reported above has either a double bond or a phenyl ring which allows the compound to obtain a reasonably stable conformation which can overlap with the three major functional groups of known competitive antagonists. Removal of the double bonds eliminates binding affinity. Alanine derivatives have reduced affinity for the receptor compared with the glycine derivatives, and isoleucine derivatives were shown to have no affinity. In each case, the α-substituent may disrupt the folding of the molecule into the preferred conformation for receptor binding.

463.17

PARADOXICAL EFFECTS OF TILETAMINE, A POTENT PHENCYCLIDINE [PCP] RECEPTOR AGONIST. T.S. Rao, J. Cler*, P.C. Contreras, S. Mick*, S. Iyengar, V. Dilworth*, J. Monahan and P.L. Wood. CNS Diseases Research, G.D. Searle & Co., St. Louis, Mo 63198.

The neurochemical effects of tiletamine [T], a PCP ligand [IC_{50} against ^{3}H-TCP, for T, MK-801 and PCP were 79 ± 2, 3.6 ± 0.6 and 44 ± 6 (nM) respectively] with weak affinity for quisqualate, kainate, glycine, dopamine [DA] D_1 and D_2 receptors, were examined on cerebellar cyclic GMP [cGMP] and rat pyriform cortical [PYR] DA metabolism. Parenteral administration of T resulted in stereotypy and ataxia, a characteristic feature of PCP receptor agonists. MK-801 and tiletamine decreased cGMP [ED_{50}, 0.35 and 4 mg/kg respectively]. While MK-801, dexoxadrol, ketamine and PCP increased PYR DA metabolism, T [2-25 mg/kg] was ineffective. T [1-10 ug/rat, icv] also did not affect PYR DA metabolism. The apparent lack of effect of T at increasing PYR DA despite its ability to decrease cGMP like other PCP agonists, tentatively suggests a selective interaction with N-methyl-D-aspartate [NMDA]-coupled PCP receptors.

463.18

(+)-N-ALLYLNORMETAZOCINE (NANM)-LIKE DISCRIMINATIVE STIMULUS EFFECTS OF N-METHYL-D-ASPARTATE (NMDA) ANTAGONISTS IN RATS. J. Willetts, A. Rice* and R.L. Balster*. Dept. of Pharmacology and Toxicology, Medical College of Virginia, Virginia Commonwealth University, Richmond, VA 23298.

The effects of the competitive NMDA antagonists, NPC 12626 (2-amino-4,5-[1,2-cyclohexyl]-7-phosphonoheptanoic acid) and CPP (3-{[±]-2-carboxypiperazin-4-yl}propyl-1-phosphonic acid), were compared to those of the non-competitive NMDA antagonists, phencyclidine (PCP) and MK-801, in male Sprague-Dawley rats trained to discriminate 5 mg/kg (+)-NANM from saline under a standard two-lever fixed-ratio 32 schedule of food reinforcement. (+)-NANM (1.25-20 mg/kg), PCP (0.1-6.0 mg/kg) and MK-801 (0.01-0.17 mg/kg) all dose-dependently substituted for the training dose of (+)-NANM in all rats tested. The order of potency for (+)-NANM lever selection was MK-801>PCP>(+)-NANM. Conversely, NPC 12626 (10-100 mg/kg) and CPP (3.0-20 mg/kg) produced no more than an average of 70% (+)-NANM lever responding at doses that reduced response rates by more than 50% of corresponding control response rates. Methohexital (5.0-17.3 mg/kg) also produced an average of 50% (+)-NANM lever responding at doses that reduced response rates. In addition to supporting a role for the PCP receptor in transducing the discriminative stimulus effects of (+)-NANM, these results lend further support for differences in the behavioral effects of competitive and non-competitive NMDA antagonists. (Supported by NIDA Grant DA-01442).

463.19

THE EFFECT OF NONCOMPETITIVE N-METHYL-D-ASPARTATE (NMDA) ANTAGONISTS ON SELECTED NEUROTRANSMITTER UPTAKE SYSTEMS. D.K. Boyd and R.D. Schwarz, Parke-Davis Pharm. Res. Div., Warner-Lambert Co., Ann Arbor, MI 48105

Behavioral effects of phencyclidine (PCP) have been attributed to its binding to a site within the NMDA receptor complex, blockade of ion channels, or modulation of neurotransmitter release and/or uptake. While other noncompetitive agents also bind to the PCP site, it is unclear what their activity is in various neuronal uptake systems. Using slices from the appropriate rat brain region, inhibition of DA (striatum), NE (cortex) and 5-HT (cortex) high affinity uptake was measured for 5 min., using tritiated ligand at 37°C. PCP was found to have IC_{50} values of 2.2μM, 0.87μM, and 5.75μM respectively. The other noncompetitive agents showed a wide range of activity with the rank order for each assay being: ^{3}H-DA: PCP < ifenprodil < MK 801 < dextromethorphan (DM) < ketamine, ^{3}H-NE: ifenprodil < PCP < MK 801 = DM < ketamine and ^{3}H-5HT: DM < ifenprodil < PCP < MK 801 < ketamine. Additionally, the competitive NMDA antagonist, 3-(2-carboxypiperazin-4-yl)propyl-1-phosphonic acid (CPP), was inactive in all assays. In conclusion, there is no consistent pattern of activity for uptake inhibition with these agents. Thus, the observed activity may not be responsible for effects common to all non-competitive NMDA antagonists (eg. antischemic efficacy, psychotomimetic side effects).

463.20

Intoxicating Concentrations of Ethanol (EtOH) Inhibit NMDA Receptor-mediated EPSPs in Adult Rat Hippocampus David M. Lovinger*, Geoffrey White* and Forrest F. Weight, Section of Electrophysiology, LPPS, National Institute on Alcohol Abuse and Alcoholism, Rockville, MD 20852 U.S.A.

We recently reported that EtOH at intoxicating concentrations inhibits the ion current activated by the glutamate receptor agonist NMDA in cultured hippocampal neurons (Lovinger et al., Science, 243; 1721,1989). We now report that EtOH inhibits synaptic transmission mediated by NMDA receptors in the CA1 sub-field of hippocampal slices from adult rats. NMDA receptor-mediated population EPSPs (pEPSPs) were isolated using the kainate/quisqualate receptor antagonist DNQX (10 μM) and low Mg^{2+} (0.1 mM). Ethanol inhibited NMDA receptor-mediated pEPSPs. Inhibition increased as the EtOH concentration increased from 1 mM (little or no inhibition) to 50 mM (43% inhibition). NMDA receptor-mediated pEPSPs were also inhibited by EtOH in the presence of the $GABA_A$ receptor antagonist bicuculline (100 μM), suggesting that the inhibition of the pEPSP is not secondary to a change in GABAergic transmission. EtOH (50 mM) did not inhibit pEPSPs mediated by non-NMDA glutamate receptors recorded in the presence of physiological Mg^{2+} concentrations (1.5 mM) and the NMDA receptor antagonist APV (50 μM), suggesting that EtOH inhibition of NMDA receptor-mediated pEPSPs does not result from general depression of glutamatergic transmission. These findings support the hypothesis that EtOH inhibition of NMDA receptor-mediated transmission contributes to intoxication.

463.21

EFFECTS OF ALCOHOLS ON THE GLUTAMATERGIC TRANSMISSION. M.T. Lima-Landman*[1], A.C.S Costa*[1,2], M.M. Froes*[2] and E.X. Albuquerque[1,2]. (Spon: L. Goldman) [1]Dept. Pharmacol., Univ. Md. Sch. Med., Baltimore, MD 21201 & [2]Lab. Mol. Pharmacol. II, UFRJ, Brazil.

Recently, ethanol (EtOH) has been reported to modify the function of excitatory aminoacid receptors (Lovinger, D.M. *et al., Science,* 243:1721, 1989; Lima-Landman, M.T. and Albuquerque, E.X. *FEBS Lett.,* 247:61, 1989). The impairment of certain brain function under EtOH intoxication and the growing evidence that links the NMDA- subtype of glutamatergic receptors to the memory and learning processes raised questions about alternative pathways for EtOH actions in the CNS. In the light of these findings, the effects of some aliphatic alcohols were studied on the NMDA and quisqualate-activated single channel currents in hippocampal pyramidal cells in culture using the patch-clamp technique in outside-out configuration. EtOH (1.74-174 mM or 0.01%-1%) had two major effects on NMDA-activated single currents: at low concentrations (1.74-8.65 mM) the probability of opening (p_{open}) increased without affecting the mean channel open time; at higher concentrations (86.5-174 mM) the p_{open} decreased with a concommitant decrease of mean channel open time. In quisqualate-activated single channel currents, EtOH (5 mM) decreased the p_{open}. This effect was enhanced with increasing EtOH concentrations. Mean channel open time of quisqualate-activated single channel currents decreased only at 500 mM EtOH. All these EtOH effects on NMDA and on quisqualate-activated currents could be reversed by washing. Using several alcohols (methanol, 1-butanol and isopentanol) we have observed a differential potency on the NMDA receptor/ion channel. These results suggest that glutamatergic transmission could be involved in some of the central effects of EtOH. (Supp: NIH NS25296, NIMH P-50MH44211, CNPq, CAPES Proc. 4987/88-2).

463.22

EFFECTS OF POLYAMINE SPIDER VENOM COMPONENTS ON [3H]GLU AND [3H]GLY BINDING TO RAT CNS MEMBRANES. M. F. Gullak*, M. J. Pagnozzi*, K. E. Richter* and E. E. Mena. (SPON: L Reynolds). Dept. Neurosci., Pfizer Central Research, Groton, CT 06340

Venoms from several species of spiders contain compounds which are characterized by a polyamine linked to an aromatic group. These compounds exert their paralytic effects on their prey by blocking glutamatergic synapses at invertebrate neuromuscular synapses. We have previously shown that these compounds are also selective for the NMDA receptor in the mammalian CNS (see also Mueller, A.L. et al., and Parks, T. N. et al., this meeting).

We examined the effects of the polyamine argiotoxins (Arg659 and 636) on [3H]Glu binding and uptake and on [3H]Gly binding in rat CNS membranes. These compounds had no effect on either Na^+-dept, Cl^--dept ,or Cl^--indept [3H]Glu binding in rat CNS membranes or on Na^+-dept [3H]Glu uptake in brain P2 fraction. However, both polyamines appeared to enhance [3H]Gly binding to forebrain membranes 2 to 4-fold when measured with a filter assay (ED50's of 22 and 43 μM, respectively). In contrast, no effect was seen using a microfuge assay. This apparent enhancement of binding did not occured with spinal cord membranes. The Kd of Gly and the IC50's of D-Ser, cycloserine, kynurenic acid and 7-Cl-kynurenic acid were identical in the presence or absence of Arg636. Additional experiments showed that Arg636 slowed both the dissociation rate and the association rate of Gly with its receptor. As a result, there was no observable effect on the Kd of Gly or on total Gly binding using a microfuge assay. Several divalent cations were able to mimic this effect of Arg636 but were not additive with Arg636. These cations were also able to displace [125I]Arg636 binding (see Mena et al., this meeting). The polyamines may block NMDA mediated responses by blocking Gly interactions with divalent ion channels at the NMDA receptor complex.

463.23

CNS BINDING SITES OF THE NOVEL NMDA ANTAGONIST, ARG-636. E. Mena, M. Gullak*, M. Pagnozzi,* D. Phillips* and N. Saccomano*. Dept. of Neurosci. and Med. Chem., Pfizer Central Research, Groton, CT 06340.

Argiotoxin636 (Arg636) is a polyamine component isolated from the venom of the spider, *Argiope aurantia* which blocks NMDA-induced elevations of cGMP in the rat cerebellum noncompetitively and NMDA-stimulated release of [3H]norepinephrine from rat hippocampus, both *in vitro*. Additionally, it antagonizes seizures induced by injection of NMDA in mice (see Seymour and Mena, this meeting). Arg636 can be distinguished from other NMDA antagonists by its unique effects on [3H]Gly binding (see Gullak et al., this meeting). We studied the interaction of [125I]Arg636 with CNS membranes to characterize further the mechanism of action of this novel NMDA antagonist.

Both radioactive and nonradioactive [I]Arg636 were synthesized from Arg636 using a chloroamine-T procedure and purified by HPLC. The structure of nonradioactive [I]Arg636 was determined by FAB-MS and NMR techniques to be the 5-iodo-2,4-dihydroxyphenyl acetic acid derivative. Nonradioactive [I]Arg636 blocked NMDA-induced elevation of cGMP with an IC50 similar to Arg636 (IC50's of 48 and 34 μM, respectively).

[125I]Arg636 bound to rat forebrain membranes with Kd and Bmax values of 11.25 μM and 28.95 pmol/mg protein (80% specific). The ability of other known polyamines and recently discovered polyamines from *Agelenopsis aperta* to inhibit binding paralled their activity as functional NMDA antagonists. No other compounds tested were able to block specific binding. However, divalent cations were potent inhibitors of this binding (IC50's of Mn, Co, Mg and Ca = 0.4 , 1, 3.4 and 5 mM, respectively). The similar effects of divalent cations and Arg636 on [3H]Gly binding (see Gullak et al., this meeting) and the effects divalent cations on [125I]Arg636 binding suggest that these polyamines may antagonize responses to NMDA by interacting with membrane ion channels.

463.24

IN VIVO NMDA ANTAGONIST ACTIVITY OF THE POLYAMINE SPIDER VENOM COMPONENT, ARGIOTOXIN-636. P.A.Seymour and E.E.Mena. Department of Neuroscience, Central Research Division, Pfizer Inc., Groton, CT 06340

Argiotoxin-636 (Arg636), a polyamine component from the venom of the orb weaving spider *Argiope aurantia*, has previously been shown to be a functional antagonist at NMDA receptors in rat brain *in vitro* (Pagnozzi , M. J. et al., Soc. Neurosci. Abs.,14:482,1988; see also Parks, T. N. et al., and Muellier, A. L. et al., this meeting). To evaluate its NMDA antagonist activity *in vivo*, Arg636 was administered to seizure sensitive, 22-23 day old DBA/2 mice for antagonism of audiogenic seizures. Arg636 produced 100% protection at doses of 10 and 32 mg/kg, s.c. and i.p., respectively. Locomotor activity studies showed that Arg636 did not significantly affect locomotor activity at these doses. Since this test is responsive to anticonvulsants of different classes, Arg636 was subsequently examined for specific antagonism of seizures induced by NMDA (56 mg/kg,i.p.). When administered 15 or 30 (s.c.), but not 60 min prior to NMDA, Arg636 significantly antagonized NMDA-induced seizures with a minimal effective dose of 32 mg/kg, s.c., indicating that Arg636 exerts NMDA antagonist activity after systemic administration *in vivo*.

463.25

POLYAMINE SPIDER TOXINS BLOCK NMDA RECEPTOR-MEDIATED INCREASES IN CYTOSOLIC CALCIUM IN CEREBELLAR GRANULE NEURONS. T. N. Parks[1], R. A. Volkmann[2*], N. A. Saccomano[2*], L. D. Artman[1*] and E. F. Nemeth[1]. Natural Product Sciences Inc., Salt Lake City, UT 84108[1] and Pfizer Central Research, Groton, CT 06340[2].

Polyamine-containing toxins in spider venoms have been reported to antagonize transmission at a variety of glutamatergic synapses (Ann. Rev. Neurosci. 12; Ann. Rep. Med. Chem. 24, 1989). We investigated the effects of synthetic toxins on NMDA-induced changes in cytosolic free calcium concentration ([Ca]i) in cultures of neonatal rat cerebellar granule neurons studied by fura-2 fluorimetry (see Nemeth and Parks, this meeting). Increases in [Ca]i in response to 10^{-5} M NMDA or L-aspartate in these cells are enhanced by 10^{-6} M glycine and antagonized by Mg^{++}, MK-801 (IC_{50}= 35 nM), AP5 (IC_{50}= 20 μM) and CPP but not by DNQX. Synthetic polyamine toxins are potent inhibitors of NMDA-induced increases in [Ca]i: Argiotoxin 659 (IC_{50}= 50 nM); Argiotoxin 636 (85 nM); Nephilatoxin (NSTX; 370 nM); Agelenopsis aperta toxins B1 (500 nM) and E (700 nM); Joro spider toxin (JSTX; 850 nM); Philanthotoxin-433 (15 μM). These data, in conjunction with evidence that polyamine toxins specifically affect NMDA-mediated transmission in rat hippocampus (Mueller et al., this meeting), suggest that these compounds constitute a new class of potent NMDA antagonist (see also Mena et al. and Seymour and Mena, this meeting).

LEARNING AND MEMORY–PHARMACOLOGY: OTHER I

464.1

EFFECTS OF PERIPHERAL ADMINISTRATION OF SUBSTANCE P AND NALOXONE ON AVOIDANCE LEARNING AND HABITUATION. C. Tomaz, P.J.C. Nogueira and M.S. Aguiar. (SPON: J.A. Ricardo). Lab. of Psychobiology, University of São Paulo, FFCLRP-USP, 14049 Ribeirão Preto, SP, Brazil.

One of us has shown that peripheral post-trial administration of substance P (SP) improves retention test performance of a single-trial inhibitory avoidance task in a dose-dependent way. In the present study, we examined the effects of SP peripheral administration on learning of three avoidance tasks and habituation. Male Wistar rats were tested in three inhibitory avoidance tasks: step-down, up-hill and alcove. Habituation was measured in an open field by recording the number of rearings during 2 min of free exploration. Training and test sessions were indentical and 24 h apart. The animals were injected i.p. immediately after the trainning trial with all possible combinations of vehicle, SP (0,5 , 5, 50, 100, 250 or 500 μg/kg) and naloxone (0,5 , 1, 5 or 50 mg/kg). Rats injected with 50 μg SP/kg had significantly longer step-down and up-hill latencies at the retention test then control animals, whereas such differences were not observed for the alcove task. Pre-treatment with naloxone produced facilitation on the up-hill and step-down tasks. In the habituation test, there were significant effects for the doses of 5 and 50 μg SP/kg, lower and higher doses being ineffective. Additionally, for this test, we included a 5 h delayed injection group, which did not differ from the vehicle control.

Supported by Brazilian National Research Council (CNPq)

464.2

PRETRAINING NALOXONE ENHANCES MORRIS WATER MAZE PERFORMANCE AND ALTERS TRAINING-INDUCED CHANGES IN HIGH AFFINITY CHOLINE UPTAKE (HACU). M. W. Decker, I. B. Introini-Collison, and J. L. McGaugh. Center for the Neurobiology of Learning and Memory and Dept. of Psychobiology, Univ. California, Irvine. CA 92717.

Posttraining administration of opiate antagonists enhances retention in a variety of learning and memory tasks. The role of opioid peptides in spatial tasks, however, is not established. We explored the role of opioid peptides in spatial learning by assessing the effects of pre- and posttraining naloxone on water maze acquisition.

Rats were given naloxone HCl (0, 1 or 3 mg/kg ip) either 5 min. before or immediately after each day's training session (2 trials/day) for 4 days. A 5th session was conducted without drug injections and was immediately followed by a free swim probe trial. Pretraining naloxone produced a dose-dependent increase in the rate of acquisition as measured both by escape latencies during training and search pattern analysis during the free swim. Posttraining naloxone had no significant effect.

In a parallel set of rats receiving daily pretraining naloxone (3 mg/kg ip), septohippocampal cholinergic activity, which is thought to be important in the acquisition of spatial information, was assessed by measuring hippocampal HACU 15 min. after the last trial on day 4. HACU was significantly reduced in place-trained rats and in swim-yoked controls relative to behaviorally naive rats. Naloxone significantly reduced HACU in behaviorally naive rats, but attenuated the swimming-induced decrease in HACU. These results suggest that naloxone alters the response of the septohippocampal cholinergic system to water maze training.

These experiments suggest that opioid peptides play an important modulatory role in the acquisition of spatial information and that they may produce some of these effects via an action on the septohippocampal cholinergic system.

Support: NIA Fellowship AG05446 (MWD), NIMH Grant MH12526 (JLM), ONR Contract N00014-87-K-0518 (JLM).

464.3

DELAYED DEVELOPMENT OF AMNESIA FOLLOWING INTRACRANIAL INJECTION OF [LEU]ENKEPHALIN IN TWO-DAY-OLD CHICKS. P.J. Colombo, J.Tsai*, E.L. Bennett, M.R. Rosenzweig and J.L. Martinez Jr. . Department of Psychology, University of California, Berkeley, CA 94720.

[Leu]enkephalin (LE) impairs retention of peck-aversion training in two-day-old chicks (Patterson et al., Behav. Neurosci., 103:429, 1989). To characterize further the development of amnesia following intracranial (IC) injection of LE, chicks were tested either 5, 15, 30, 45, 60, 90, 120, 180, 240 min, or 24 hr after training. Chicks injected bilaterally in the region of the intermediate medial hyperstriatum ventrale with LE performed like saline injected controls during training as measured by latency to peck and number of pecks. LE injected chicks performed like controls when tested at each of the post-training time points between 5 and 180 min. At 240 min post-training, LE -treated chicks show a moderate impairment, and are amnesic when tested at 24 hr. Previous investigation of chicks indicates that amnesia develops within one of three temporal courses. The current findings suggest that IC injection of LE produces amnesia with novel temporal characteristics. Also, B-endorphin was found to produce amnesia more rapidly than other agents studied in our laboratory (Bennett, et al., Soc.for Neuroscience, 1988). These results suggest that opioids may affect memory formation through mechanisms other than those previously studied.

464.4

ENHANCEMENT OF MEMORY FOR A ONE-TRIAL PASSIVE AVOIDANCE TASK IN CHICKS GIVEN PERIPHERAL AND CENTRAL INJECTIONS OF NALOXONE. D.W. Lee, M.K. Means*, D.J. Afinowicz*, E.L. Bennett, J.L. Martinez Jr., & M.R. Rosenzweig. Dept. of Psychology, Univ. of California, Berkeley, CA, 94720.

To investigate memory enhancement for a one-trial passive avoidance task in chicks, a weak concentration (10%) of the bitter tasting liquid methylanthranilate (MeA) was used as the aversive stimulus. Naloxone (Nal), an opioid receptor antagonist known to generally enhance some forms of memory, was without effect when 100% MeA was used. Several experiments were conducted administering central (i.c.) bilateral injections directed to the region of the medial hyperstriatum ventrale or peripheral (i.p.) injections of Nal or saline (Sal). The effective doses for each injection route were determined by injecting either Sal or Nal i.c. or i.p. 5 min pretraining. When tested at 24 hrs, intermediate doses for both routes resulted in the highest levels of retention (u-shaped curves). For i.c., 60-96 nmol/chick and for i.p., 1000 nmol/chick (10 mg/kg) Nal were the most effective doses resulting in strong enhancement effects (80-85% retention) when compared to Sal (40-43% retention). The effective dose i.c. (96 nmol) also significantly enhanced retention when injected 5 min posttraining as well as 5 min pretraining. Central injections were 10 times more potent than peripheral injections. These results: 1) show a central action of Nal, 2) suggest that the apparent peripheral action may be central, but 3) do not rule out a peripheral action.

Supported by NIDA grant DA-04795-02 and NSF grant BNS-88-10528.

464.5

EFFECTS OF POSTNATAL TESTOSTERONE PROPRIONATE ADMINISTRATION ON MORRIS WATER MAZE PERFORMANCE IN RATS. R.L. Roof. Dept. of Psychology and Neuroscience Program, University of Wyoming, Laramie, WY, 82071.

To study the involvement of testosterone in the development of spatial abilities, male and female rats were injected with either 75 ug or 150 ug Testosterone Proprionate (TP) or an oil vehicle on days 4 and 6 after birth. At 90+ days of age, the rats were trained and tested on the Morris water maze (MWM). Rats were tested once a day for 6 successive days, and were retested after a 3 week delay. TP effects on MWM performance were observed for the trial at which the first significant drop in time and distance required to reach the platform occurred. TP treated females improved more quickly than control females, and control males improved more quickly than TP treated males. In addition, control males required less time than control females to reach the platform on 4 of the 6 initial trials. There were no differences between the groups on the trials after the delay.

These findings support the hypothesis that steroid hormones have an organizational effect on the developing brain that subsequently influences cognitive functioning.

464.6

EFFECTS OF FORSKOLIN, CAFFEINE, AND CYCLOHEXIMIDE ON MEMORY RECALL AFTER APPETITIVE CONDITIONING IN THE HONEY BEE. B.H. Smith, P.W. Hamlet, A.E. McNair and T.R. Tobin. ARL Div. of Neurobiology, Univ. of Arizona, Tucson AZ 85721.

Models of memory consolidation invoke a multi-stage process wherein the different stages are dependent upon specific biochemical mechanisms. Recall at intermediate intervals after associative conditioning may be dependent upon mechanisms involving cAMP, whereas longer term responding is thought to involve gene activation and protein biosynthesis. Using associative conditioning of the proboscis extension reflex of the honey bee, we undertook to determine time windows for sensitivity of recall to different substances that modulate these biochemical mechanisms.

Restrained honey bee workers were conditioned to extend their mouthparts to an odorant by 1 to 4 forward pairings (30 sec ITIs) of that odorant with a 2.0M sugar water reward. All bees were tested with a *single* extinction trial at one of several times ranging from 5 min to 120 min post-conditioning. In a control series to determine long-term recall levels, responses were equivalent among bees conditioned with 1, 2, 3, or 4 trials at all times through 120 min and involved associative and non-associative behavioral mechanisms.

In several subsequent experimental series, each bee received an abdominal injection containing either saline, or one of several concentrations of forskolin, caffeine, or cycloheximide in the same saline. Bees injected with caffeine or forskolin 45 min prior to a single conditioning trial had similar levels recall to bees injected with saline alone when tested at either 45 min or 120 min post-conditioning. Bees injected with cycloheximide 30 min prior to conditioning had decreased levels of recall 45 min and 120 min after conditioning with 4 trials. However, effects were mixed in groups injected 180 min prior to conditioning. Biochemical characterization of protein synthesis in these groups of bees may elucidate these phenomena.

464.7

THE INTERACTIVE EFFECTS OF CAFFEINE AND PHASE OF MENSTRUAL CYCLE ON FREE RECALL. M. E. Arnold*, B. E. Beckwith and T. V. Petros. Psychol. Depart., Univ. of North Dakota, Box 7187 Univ. Sta., Grand Forks, ND 58202.

The effect of caffeine on memory in women who were and were not taking oral contraceptives was examined. Thirty minutes after ingesting either 0, 2 or 4 mg/kg of caffeine at days 1-5 or days 9-13 of their menstrual cycle, subjects listened to lists of words and provided immediate free recall of each list along with a final free recall of all the lists. Caffeine facilitated recall of words in the primary positions of the lists and women on oral contraceptives recalled fewer primacy words than pill-free women. There were also complex interactions between treatment, phase of menstrual cycle, practice and rate of presentation of words. During the final recall, caffeine enhanced overall recall. Caffeine enhanced recall for those on oral contraceptives during the early but not the second phase of the menstrual cycle. These results suggest the need for further study of neuroendocrine modulation of the effects of caffeine.

464.8

EFFECTS OF PSYCHOACTIVE DRUGS ON LEARNING AND MEMORY AS ASSESSED USING A NOVEL WATER MAZE. G.J. Kant, C.P. D'Angelo*, A.J. Brown*, C. Myatt*, E. Lewis*, T. Robinson* and T. Eggleston*. Dept. of Medical Neurosciences, Walter Reed Army Institute of Research, Washington DC 20307.

We have recently reported on the usefulness of a novel water maze for the assessment of learning and memory not confounded by possible effects of the drug or physiological variable on appetite [Kant et al., Pharmacol. Biochem. Behav., 31:487,1988; Przybelski et al., Biomat Artif Cell Artif Organs, (in press)]. The maze consists of three sets of 4 walls arranged in concentric squares set inside a 5 ft dia. pool. Each of the walls has a door that can be opened or closed providing a variety of possible mazes of varying difficulty. In the present study, two groups of rats (10 rats/group) were trained on one configuration of the water maze. In subsequent testing, one group (B) was always tested on the same "training" maze while the other group (A) was tested on a different maze configuration of approximately the same difficulty each day. Each test day consisted of 3 timed trials begun 15 min following drug injection. Both time to finish and errors committed were recorded for each trial. The 3 trials were separated by 30 sec rest periods. Not surprisingly, the B group completed the maze each test day in less time and with fewer errors than did the A group rats, even when the A group was tested on the identical maze as the B group. Both groups, however, were sensitive to the disrupting effects (increased maze time and/or more errors) of diazepam, amphetamine and caffeine, while atropine did not disrupt maze performance.

464.9

RELATIONSHIP OF HIPPOCAMPAL PROTEIN KINASE C (PKC) ACTIVITY TO SPATIAL LEARNING PERFORMANCE. J.M. Wehner, S. Sleight* and M. Upchurch. Inst. for Behavioral Genetics, Univ. Colorado, Boulder, CO 80309.

C57BL/6 mice perform the Morris water task for spatial learning well while DBA/2 mice perform poorly (Upchurch and Wehner, Beh. Gen.18: 55, 1988). We have studied potential neural substrates that may mediate this difference. PKC activity was measured in cortex and hippocampus. C57BL had greater lipid-, and Ca^{++}-stimulated activities than did DBA/2 mice. Because this difference may be fortuitous and unrelated to learning ability, 11 C57BL X DBA/2 recombinant inbred strains (RIs) were assessed for spatial learning ability in the Morris water task and cortical and hippocampal tissue assayed for basal and stimulated PKC activity. Behaviorally, the RIs ranged in learning ability from those that resembled DBA mice to those superior to either parental strain. PKC activities also varied in cortical and hippocampal tissue. Significant correlations between hippocampal phosphatidyl serine-stimulated activity and a site-crossing preference score ($r=.80$, $P<.01$) or a search-time preference score ($r=0.7$, $P<.02$) were observed in these RIs. No significant correlation was observed for cortical PKC activity and any learning measure. These data support the conclusion that hippocampal PKC is associated with spatial learning performance as measured by the Morris test. (supported by NSF #BNS-8820076).

464.10

CHARACTERISTICS OF HIPPOCAMPAL COMPLEX SPIKE NEURONS DURING PERFORMANCE OF A DELAYED MATCH TO SAMPLE TASK. SELECTIVE INFLUENCES OF DELTA-9-THC. C.J. Heyser*, R.E. Hampson and S.A. Deadwyler Dept. of Physiology & Pharmacology, Bowman Gray Sch. of Med., Wake Forest Univ., Winston-Salem, NC 27103

Specification of hippocampal involvement in memory processes requires a precise description of the manner in which hippocampal cells participate in the coding and retrieval of sensory information. The delayed match to sample (DMTS) paradigm can be used to examine the participation of hippocampal cells in retention of sensory information. We examined the response characteristics of identified hippocampal complex spike cells in a DMTS task in which performance decayed from 92 to 70% mean correct responses over delay intervals ranging from 1-31 sec. Both the temporal and spatial correlates of hippocampal complex spike cell activity were investigated and characterized. Prior studies have shown that delta-9-THC, the psychoactive ingredient in marihuana, disrupts performance at longer but not shorter delays in the DMTS task, closely resembling the effects of hippocampal lesions. Results indicate that 90% of complex spike cells encountered in the dorsal hippocampus responded just after execution of the match response; 56% fired just prior to the sample press and just after the match response, and less than 10% of the cells fired prior to the sample press only. THC (2.0 mg/kg, IP) eliminated the response to the sample lever but not the match lever in all cells. THC did not alter the match cell discharge in any group of cells tested. These results suggest that the dissociation of the sample- and match-provoked cell discharges by delta-9-THC was responsible for the significant disruption of behavioral responding in the DMTS task. Possible cellular actions of THC on hippocampal pyramidal cells are discribed in terms of ongoing in vitro studies. [*Supported by Grants DA04441, DA03502, and KO5-DA00119 to S.A.D.*]

464.11

SLEEP AND MEMORY DEFICITS IN AMYGDALA-KINDLED RATS: EFFECTS OF GLUCOSE. W.S. STONE, R.J. RUDD, M. SMITH & P.E. GOLD. Dept. Psychol., Univ. Virginia, Charlottesville VA 22903.

Extensive amygdala kindling results in deficits in sleep and in inhibitory avoidance tests. Individual differences in sleep and memory deficits significantly correlated in these animals. The present study demonstrates that deficits in spontaneous alternation (SA) are also produced by amygdaloid kindling and are correlated with changes in sleep. Glucose, which improves memory in other amnestic conditions, attenuates kindling-induced alterations in SA. Male Sprague-Dawley rats had electrodes implanted in neocortex and in basolateral amygdala. After recovery, 3-hr baseline sleep samples were obtained. One group received daily kindling stimulation (biphasic square waves, 1 msec, 60 Hz, 250 uA, 1 sec train) for 4-5 weeks, while another group served as non-stimulated controls. One week after the last seizure, sleep records were again obtained. All rats were then tested for SA performance (20 trials, 1 min ITI) 30 min after glucose (100 mg/kg) or saline injection.

Compared to the implanted controls, kindled rats showed decreases in bout number and duration of both paradoxical and nonparadoxical sleep. SA performance was also significantly impaired. Individual differences in several sleep measures were significantly correlated with SA performance in kindled rats. Glucose administration improved SA performance to the level of the non-kindled control group. The results demonstrate relationships between sleep and memory deficits in kindled rats, and suggest that glucose might be useful in attenuating some forms of seizure-induced memory deficits. (Supported by NIMH 31141, ONR N00014-85-KO472, and NRSA AG 05408).

464.12

NO EVIDENCE FOR TIME-LOCKED EFFECTS OF EMETINE INJECTED INTO THE MHV OF THE CHICK BRAIN. P.A. Serrano, S.J. Ramus*, E.L. Bennett and M.R. Rosenzweig. Department of Psychology, University of California, Berkeley, CA 94720

Gibbs & Ng (1977) have proposed a three-stage model for the formation of memory in the 2-day-old chick. Each of the three stages, short-term, intermediate-term, and long-term memory, appear to depend upon a particular neurochemical event. Each stage is characterized by the time at which amnesia appears after training on a passive avoidance task. A protein synthesis inhibitor, emetine, disrupts long-term memory and exhibits its effects on memory 90 min after training (Patterson et al., 1986). To investigate whether the amnesia from emetine represents a time-locked effect of the drug's action in the brain, a time course for the appearance of amnesia was conducted using two different pre-training injection times: 5 min and 20 min.

Two-day-old chicks were given bilateral injections of 4.0mM emetine or saline into the region of the medial hyperstriatum ventrale (MHV). The MHV is an area of the chick brain where metabolic activity is elevated as a result of learning in both a passive avoidance task (Rose et al., 1986) and in imprinting (Horn et al., 1979). After injection, groups of chicks were trained using a one-trial passive avoidance task either 5 min or 20 min later. Subsequent tests were done at 60, 75, 90, 105 min, and 24hr. The results show that emetine produces amnesia at 90 min in both the 5 min and 20 min pre-training injection groups without recovery of the memory. This suggests that the amnesia seen from the injections of emetine are the result of inhibition of the formation of long-term memory and not an artifact of the time required for the drug to disrupt the memory. Supported by NSF grant BNS-86-06938.

464.13

REVERSAL LEARNING IN RATS: CLASSICAL VERSUS INSTRUMENTAL CONDITIONING PARADIGMS. P.J. Bushnell. Neurotoxicology Division, U.S. EPA, Research Triangle Park, NC 27711.

Reversal learning set formation provides a useful model for the study of complex learning in animals. Traditional methods have relied upon single-response instrumental discriminations with their concomitant limitations. Spatial reversal learning set formation was explored in Long-Evans rats using a two-lever automaintenance (AU) procedure, in which one lever (CS^+) was paired with food reward and the other (CS^0) was not. Reward was provided on every trial regardless of the responses emitted. Behavior of rats trained on the AU task (n=6) was compared to that of rats trained on a similar instrumental (IN) task (n=7), in which at least one response to the CS^+ was required for reward on each trial. Response accuracy was calculated as a discrimination ratio DR = CS^+ responses per 10 trials/ total responses per 10 trials. Overall response rates were higher in the IN group than in the AU group, but both groups acquired the original spatial discrimination (CS^+ = right, CS^0 = left) at equivalent rates. Both groups formed similar reversal learning sets, requiring fewer trials to reach criterion (DR $\geq$ 0.9 for 2 10-trial blocks) across consecutive reversals. Three of 7 IN rats extinguished during acquisition of R2; their performance was reinstated by 2 sessions of remedial training on the AU schedule. Reversal (R) was faster in the IN group on R1, but slower after the learning set had been acquired (R11 to R16). Fitting 2-parameter nonlinear functions to acquisition curves showed that, while asymptotic discrimination accuracy did not differ, rate of acquisition was faster in the AU group (0.13$\pm$0.02) than in the IN group (0.05$\pm$0.003). These results show that spatial reversal learning sets can be formed in an automaintenance environment, in which reward does not depend upon accurate responding.

464.14

STRAIN DIFFERENCE BETWEEN C57BL/6IBG AND DBA/2IBG MICE IN A SPATIAL WORKING MEMORY TASK. M. Upchurch, L. Wurtz* and A. Cuestas*. Psychology Dept., Benedictine Coll., Atchison, KS 66002.

Previous studies have indicated that C57BL/6Ibg mice differ from DBA/2Ibg mice in spatial learning ability in the Morris water task, a test of spatial reference memory. A spatial working memory task in a Y-maze was used to test for the generality of the spatial learning difference between the two strains. Male mice were trained to go down the arms of the Y-maze for a food reward. During a pre-training phase, animals learned that all arms of the maze contained food and that food was not replaced once it was eaten. Following pretraining, mice were trained either in a spatial match-to-sample (MS) or a spatial nonmatch-to-sample (NMS) task. For MS mice, the correct response was to turn in the same direction that they had taken during a forced-choice sample trial. This choice took them into a previously unvisited arm. For the NMS mice, the correct response was to turn down the arm that they had originally started from and that had not contained any food. C57 mice performed better than DBA mice at the MS task and were substantially worse than DBA mice at the NMS task. The results suggest that C57 mice had formed a cognitive map of maze locations where food was likely or unlikely to be. These results are consistent with earlier work on the spatial learning ability of these two strains of mice in the Morris water task.

464.15

LEARNING AND MEMORY IMPAIRMENT IN RATS FED A HIGH SATURATED FAT DIET. G. Winocur* and C.E. Greenwood (SPON: G.H. Anderson). Dept. Psychology, Trent University, Peterborough, Ont., K9J 7B8 and Dept. Nutritional Sciences, Univ. Toronto, Toronto, Ont., Canada M5S 1A8.

Our previous studies indicate that alterations in the type of dietary fats fed to rats influence a variety of behaviors, including learning and memory performance. To conduct a more systematic investigation of cognitive performance, three groups of male Long Evans rats (one month of age) were fed 20% (w/w) fat diets high in either saturated fatty acids (lard-based) or polyunsaturated fatty acids (soybean oil (SBO)-based) or laboratory chow (4.5% (w/w) fat with similar fatty acid profile to SBO). After three months, all rats were tested on Olton's radial arm maze, a variable-interval delayed alternation task (VIDA) and the Hebb-Williams maze series. The lard-fed group was impaired on all tests. The SBO group was slightly impaired on some measures, relative to the chow-fed group, but consistently performed better than the lard-fed group. The results suggested that the impact of dietary fat on performance was not confined to one brain region; that is, performance was altered to a greater extent when tasks required increased integration from a number of brain regions (eg. VIDA and Hebb-Williams mazes).

As a first step towards elucidating the physiological mechanism whereby fat is influencing brain function, whole brain regional phospholipid (PL) fatty acid profiles were determined using GLC with flame ionization detection. Regional differences in fatty acid profiles were observed, probably reflecting the distribution of myelin amongst regions and its different fatty acid profile in comparison to other subcellular membranes. Dietary fat composition influenced PL fatty acid profile, with phosphatidylethanolamine being more affected than phosphatidylcholine. No diet by region interaction was observed, suggesting that all regions were affected similarly. Collectively, these results suggest that dietary fat has a modest impact on neuronal function within specific regions. Neuronal membrane compositional changes are observed concomitantly with functional change and these changes are regionally nonspecific in agreement with the behavioral data. (NSERC and MRC).

464.16

EFFECTS OF VARYING TRAINING STRENGTH ON SHORT-, INTERMEDIATE-, AND LONG-TERM FORMATION OF MEMORY (STM, ITM, LTM) FOR A ONE-TRIAL PASSIVE AVOIDANCE TASK IN CHICKS. M.R. Rosenzweig, D.W. Lee, M.K. Means*, E.L. Bennett, J.L. Martinez Jr. Dept. of Psychology, Univ. of California, Berkeley, CA, 94720.

Upon presentation of a small metal bead dipped in 100% methylanthranilate (MeA), a bitter tasting liquid, chicks will peck the bead, taste the bitter liquid, then subsequently avoid a similar but dry bead presented at test. Test can be anytime from 10 s to 24 hrs after training. Gibbs & Ng (1977) reported that memory of chicks trained in this 1-trial passive avoidance task shows "dips" (lower percent avoidance) at about 12 and 55 min posttraining and suggest that these dips mark the transitions between STM & ITM and between ITM & LTM. The existence of two sharp dips has not yet been replicated. Since 80-95% of chicks avoid the bead at most test times, this task may result in a ceiling effect possibly obscuring more subtle aspects of memory formation. Studies with strong and moderate peck aversion training of chicks were undertaken both to explore the possible occurrance of transitional dips and to conduct studies using memory enhancing drugs.

Two-day-old chicks were injected with 10 µl saline. Five minutes later, they were trained by dipping the bead in either 5%, 10% or 100% MeA. Testing occurred at times from 10 s to 24 hrs after training. Diluted MeA results in progressively weaker training and retention: at every test time, chicks trained with the 100% MeA showed greater avoidance (90% at 24 hrs) than those trained with 10% MeA (40%) or those given 5% MeA (32%). Our results also show dips around 1, 15 and 60 min for both the 5% and 10% MeA groups supporting the notion of successive stages in memory formation.

Supported by NSF grant BNS-88-10528 and NIDA grant DA-04795-02.

465.1

EFFECTS OF BUSPIRONE AND ALPRAZOLAM ON A THREE-CHOICE WIN-STAY WATER-ESCAPE WORKING MEMORY TASK IN RATS. E.W. Bass*, B.A. McMillen and L.W. Means. Depts. of Psychol. & Pharmacol., East Carolina Univ., Greenville, NC 27858.

Two studies were conducted to determine if buspirone (BUS) or alprazolam (ALP) would either improve performance on a working memory water-escape task by reducing perseverative responding or impair performance by interfering with memory processes. Both studies involved giving rats 3 daily test trials, each trial consisting of an information run during which guillotine doors forced the rats to swim into the correct escape alley and a test run during which the rats could enter any of 3 alleys, but escape only upon entering the same alley to which they had been forced. In the first experiment, rats were trained to a 70% correct choice criterion with 5 min inter-run intervals and then tested for performance with 5, 20 and 60 min inter-run intervals. Rats injected 1 hr before testing with BUS (3mg/Kg s.c.) were significantly ($p<.05$) impaired relative to rats injected with vehicle, while rats injected with 0.5mg/Kg ALP did not differ significantly from controls. In a second experiment, rats in each of the drug groups were tested after receiving 1 of 3 different doses of their respective drug. All three doses of BUS (1,3,10mg/Kg) impaired performance ($p<.05$) while none of the doses of ALP (0.5,1,2mg/Kg) impaired performance. Thus, BUS impaired performance of rats on a water-escape working memory task. (Supp. by DA-04895)

465.2

CHLORDIAZEPOXIDE AND dl-PROPRANOLOL PRETREATMENT OF CONDITIONED HYPERGLYCEMIA. P. S. Grigson and C. F. Flaherty. Psychology Department, Rutgers University, New Brunswick, NJ 08903.

A history of insulin administration in a novel environment leads to a conditioned hyperglycemic response in rats when injected with placebo on the test day for conditioning. This hyperglycemic response is reversed to hypoglycemia when pretreated with chlordiazepoxide (CDP) during both the conditioning and test phase (Flaherty, et al., Physiol & Behav., 33:595-599, 1984). The present study was concerned with the further investigation of CDP and the beta-adrenergic blocker dl-propranolol on glycemic conditioning. Two groups were pretreated with CDP (10 mg/kg ip) either during conditioning or on the test day only. Three additional groups were pretreated with propranolol (5 mg/kg ip) during the conditioning phase only, on the test day only or during both the conditioning phase and on the test day. The results indicated that a history of insulin administration in a novel environment tended to lead to conditioned hyperglycemia, and this tendency was accentuated by propranolol pretreatment. There was no evidence for conditioning in the CDP pretreated groups - possibly due to generalization decrement or state-dependent learning.

465.3

FLUMAZENIL REDUCES POSTOPERATIVE AMNESIA AFTER MIDAZOLAM ANESTHESIA IN AMBULATORY SURGERY PATIENT. Y.F.Sung, M.D., Co-Investigator: N. Reiss, CRNA*. Department of Anesthesiology, Ambulatory Surgery Center, The Emory Clinic, Atlanta, GA 30322.

Benzodiazepines are known to induce anterograde amnesia - an action which may be desired in certain situations, e.g.: during preoperative and operative sedation, induction for general anesthesia, and maintenance for general anesthesia. Midazolam hydrochloride has anxiolytic and amnestic properties that are similar to those of other benzodiazopines, however, it is unique in that it is water soluble and does not require a solvent that may cause venous irritation during injection. The present report is from a double-blind study performed at our institution to assess the ability of flumazenil, a benzodiazepine antagonist, to reverse the postoperative amnesia after midazolam anesthesia. Midazolam resulted in anterograde amnesia. The patient could not recall picture shown after they received the drug.

In comparison to placebo, flumazenil significantly improved the recall postoperatively after midazolam anesthesia.

465.4

EFFECT OF DIAZEPAM, TRIAZOLAM AND Ro 15-1788 ON CLASSICAL CONDITIONING. J.A. Harvey, A.G. Romano & V. Smith. Med. College of Pennsylvania at EPPI, Philadelphia, PA 19129.

Three experiments were carried out to examine the effects of two benzodiazepines on associative learning as measured by acquisition of the rabbit's nictitating membrane response. In a first study, conditioning was accomplished by pairing light and tone CSs with a shock US. Diazepam (0.1, 0.3 and 1.0 mg/kg) produced a dose dependent reduction in the acquisition of CRs to both tone and light CSs. The dose of diazepam needed to block criterion acquisition in 50% of the animals was calculated to be 0.145 mg/kg. A second study, employing explicitly unpaired presentations of CSs and US, indicated that diazepam had no effect on baseline responding or on responding to the tone and light CSs but did produce a dose-dependent reduction in the amplitude of the UR elicited by the shock US. In a third study, conditioning was carried out through the pairings of a tone CS and an air puff US. Triazolam (0.05 mg/kg) retarded CR acquisition, while the benzodiazepine antagonist, Ro 15-1788 (3 mg/kg), had no effect. However, Ro 15-1788 completely prevented the retardation in CR acquisition produced by triazolam. It was concluded that diazepam and triazolam were retarding associative learning through an action on the benzodiazepine receptor and that part of this retardation might be due to an effect on the unconditioned reflex. Supported by NIMH Grant MH16841.

465.5

FLUMAZENIL (Ro 15-1788) IMPROVED LEARNING AND MEMORY IN MICE BUT NOT IN MONKEYS. L. Rumennik, G.P. Vincent, E. Schwam and J. Sepinwall. Department of Neurobiology and Obesity Research, Hoffmann-La Roche Inc., Nutley, N.J. 07110.

The benzodiazepine (BZ) antagonist flumazenil (F) was reported to improve learning and memory in mice at doses ranging from 2.5-40 mg/kg, i.p. (Lal, H. et al., FASEB J. 2:2707, 1988). We evaluated the cognitive effects of this compound in CF1 mice and squirrel monkeys. In an active avoidance procedure, 40 mg/kg, i.p. improved 24 hr. retention in mice when administered 10, but not 30, min. before the training session. This dose also enhanced retention when given immediately following the training session, suggesting an enhancement of memory consolidation. In contrast, in a delayed match-to-sample procedure (DMTS) in monkeys, 1-30 mg/kg, i.g. failed to improve short-term memory (0-182 sec). Similarly, when evaluated in a repeated acquisition procedure (RA) in monkeys, 3-30 mg/kg, i.g. failed to improve learning, with impairment of learning evident at 30 mg/kg. In separate studies in monkeys, 3 and 10 mg/kg, i.g. antagonized the disruptive effects of 0.04 and 0.08 mg/kg, i.g. triazolam on learning (RA) and memory (DMTS). Thus, F reliably antagonized triazolam, but it did not consistently improve cognition; enhancement was dependent upon dose, species, and experimental paradigm.

465.6

DOSE DEPENDENT REVERSAL OF DIAZEPAM-INDUCED DISCRIMINATION IMPAIRMENT BY RO 15-1788. S.O. Cole. Dept. of Psychology, Rutgers University, Camden, NJ 08102.

Diazepam (DZ) 4 mg/kg, administered on eight successive acquisition sessions, impaired a light-cued, successive discrimination in male Sprague-Dawley rats with the animals recovering on three post-drug vehicle sessions. The impairment in discrimination was accompanied by an increase in responding during no go periods of the task, indicating that DZ-drugged animals have difficulty withholding incorrect responses. The benzodiazepine (BDZ) receptor antagonist Ro 15-1788 (5 and 10 mg/kg) reversed the discrimination impairment and reduced the number of incorrect responses in a generally dose-dependent manner when co-administered with DZ. These findings suggest that the impairment of successive discrimination by DZ is mediated by central BDZ receptor sites. When administered alone, the 10 mg/kg dose of Ro 15-1788 (but not the 5 mg/kg dose) produced a significant BDZ-like impairment in discrimination, which was accompanied by a significant increase in incorrect responses. These findings suggest that Ro 15-1788 is not a neutral BDZ-receptor antagonist but may have some intrinsic action of its own which needs to be assessed independently of its use as a mediational research tool.

465.7

SELECTIVE IMPAIRMENTS IN EXPLICIT MEMORY PROCESSES ASSOCIATED WITH ACUTE ALCOHOL INTOXICATION. R.G. Lister, C. Gorenstein*, D. Risher-Flowers*, H.J.Weingartner* and M.J. Eckardt. Lab. Clinical Studies, NIAAA, DICBR, Bethesda, MD 20892.

The effect of alcohol on learning and memory was assessed in independent groups of student volunteers (total n=28). Subjects were shown a list of words and asked to form an image of a scene involving each word 90 min after drinking a mixture of alcohol (0, 0.3 or 0.6 g/kg) and tonic water. Following a distractor task, they recalled as many of the words as possible. After free recall, they were asked to read a number of words that had been typed backwards. Some of these words were from the list that had been imaged and some were new words. Finally, subjects performed a word completion task in which a number of words were presented with letters missing. They were asked to complete each word with the first response that came to mind. The words were either; words that had been imaged; words that had been read backwards; words that had been both imaged and read backwards; or new words. Alcohol caused a dose-related impairment in free recall ($p<0.02$). In the backwards reading test, both placebo- and alcohol-treated subjects read words faster if they had previously imaged them ($p<0.001$). In the word completion task, all groups completed more words if they had either read them backwards or imaged them previously ($p<0.001$). These priming effects did not differ across the groups. The data suggest that alcohol impairs performance in tests of explicit memory but may not impair memory for the same material when tested implicitly.

465.8

DISRUPTION OF TASTE AVERSION LEARNING BY PREEXPOSURE OR TOLERANCE TO ETHANOL AND OTHER DRUGS. B. M. Rabin and W. A. Hunt*. Behavioral Sciences, Armed Forces Radiobiol. Res. Inst., Bethesda, MD 20814-5145; Dept. of Psychology, Univ. of Maryland, Baltimore, MD 21228.

Hunt and Rabin (Life Sci, 43: 59, 1988) have shown that making rats tolerant to ethanol, in contrast to ethanol preexposure, disrupts the acquisition of a radiation-induced taste conditioned aversion (CTA). Additional studies have shown that there are some residual effects from tolerance on the CTA learning for up to 5 weeks following the termination of the ethanol intubation. Similarly, ethanol tolerance, but not preexposure, disrupts the acquisition of a CTA following injection of 1.5 mEq/kg, but not 3 mEq/kg, lithium chloride. Preexposure to diazepam or to morphine does not attenuate either radiation-or lithium chloride-induced CTA learning. However, tolerance to diazepam does attenuate CTA learning following exposure to ionizing radiation or lithium chloride.

465.9

VEHICLE INFUSION INTO THE BASAL FOREBRAIN PRODUCES TASK-SPECIFIC COGNITIVE DEFICITS IN THE RAT. JJ Chrobak, D An*, & TC Napier, Department of Pharmacology, Loyola University Medical Center, Maywood, IL 60153.

Basal forebrain nuclei, the ventral pallidum/nucleus basalis (VP/NB) and medial septum (MS), are the source of cholinergic neurons implicated in cognitive processes. Intraseptal infusion of the GABA agonist muscimol produces an impairment in radial arm maze (RAM) performance. The following study examined this phenomenon within the VP/NB. Rats, trained to perform a RAM task with a 1 hr delay inserted between the fourth and fifth arm choice, recieved vehicle (saline, artificial CSF), muscimol or sham injections into the VP/NB. Vehicle and muscimol infusions, but not sham, produced similiar impairments. Deficits persisted for two days; the largest decrement being observed 24 hrs post-infusion. Baseline performance was re-established with further daily testing. A vehicle-induced impairment was not observed in rats tested on a standard RAM task with no delay imposed. Thus, the deficit was dependent upon the mnemonic demands of the task. These findings: 1) indicate that vehicle infusions into the VP/NB can cause a neural perturbation that becomes manifest in particular cognitive testing paradigms; 2) support findings that vehicle infusions into the NB can produce biochemical and behavioral alterations; and, 3) highlight the importance of uninjected controls in protocols that involve treatments within the VP/NB.

465.10

MUSCIMOL INJECTIONS IN THE MEDIAL SEPTUM IMPAIR SPATIAL LEARNING. J. D. Brioni*, M. W. Decker, L. P. Gamboa*, I. Izquierdo, and J. L. McGaugh (SPON: J. L. Davis). Center for the Neurobiology of Learning and Memory and Dept. of Psychobiology, Univ. California, Irvine. CA 92717.

This study examined the role of GABA in modulating septohippocampal cholinergic influences on memory. Microinjections of the GABA-A agonist muscimol (0.5, 1.0 or 5.0 nmol) or physiological saline were administered (0.5 µl) into the medial septum of rats via chronic implanted cannulae 5 min. prior to each of 4 daily training sessions (3 trials per session) on the Morris water maze. Rats were trained to find a submerged platform located in a fixed position in a circular pool of water. A drug-free probe trial with no platform available was conducted 4 days after the last training trial. High affinity choline uptake (HACU), an index of cholinergic activity, was also measured in a separate group of rats 5 min. after intraseptal injections of these same doses of muscimol.

Analysis of escape latencies during training revealed that learning was significantly impaired in rats given pretraining muscimol injections into the medial septum at doses (1.0 and 5.0 nmol) that significantly reduced hippocampal HACU. A lower dose of muscimol (0.5 nmol) had no significant effect on either performance or hippocampal HACU. Analysis of the search patterns during the probe trial revealed that, in comparison to controls, animals that had received either of the two higher doses of muscimol prior to each training session were less likely to swim in the region where the platform had been located.

Our results are consistent with the view that septal GABAergic modulation of the septohippocampal cholinergic pathway is involved in regulating the acquisition of spatial information.

Support: NIA Fellowship AG05446 (MWD), NIMH Grant MH12526 (JLM), ONR Contract N00014-87-K-0518 (JLM).

465.11

BICUCULLINE METHIODIDE IN THE MRF DOES NOT AFFECT LONG-TERM HABITUATION OF THE ACOUSTIC STARTLE RESPONSE. Wesley P. Jordan and Hilary Donovan*. Psychology, St. Mary's College of Maryland, St. Mary's City, MD 20686.

Short-term habituation of the acoustic startle response in the rat occurs within the response circuit, but long-term habituation is produced by an accruing inhibition of the reflex by neural activity in an separate, long-term habituation pathway. The mesencephalic reticular formation (MRF) is a part of the long-term habituation pathway because lesions there severely attenuate long-term habituation of acoustic startle. This study investigated the role of gamma-amino-butyric acid (GABA) within the long-term pathway. Drugged rats (n=8) received 50ng of bicuculline methiodide (BMI) to each side of the MRF 10-min before each of 5 test sessions. The 5 control animals received saline. BMI affected motor activities and depressed startle amplitudes, precluding a clear determination of habituation. Control rats habituated over sessions. A retest without injections found the startle amplitudes of controls and formerly drugged animals were at the long-term habituation asymptote of the controls, suggesting that long-term habituation had occurred during the drug sessions. Animals subsequently habituated normally to a tactile stimulus presented without drug injections, suggesting no lingering effects of the drug on startle habituation. GABA does not appear to be released at MRF synapses within the long-term pathway, but could be at other sites within the pathway.

465.12

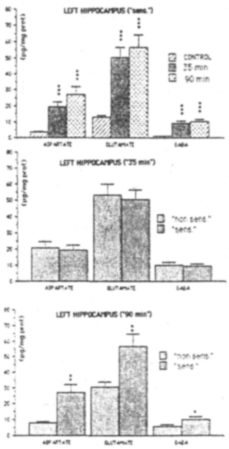
DIFFERENTIAL EFFECTS OF BACLOFEN ON ACQUISITION IN POSITIVE VS. NEGATIVE TRANSFER OF DISCRIMINATION TESTS. D.B. Clissold*, S. J. Enna, and M. J. Pontecorvo. CNS Pharmacology, Nova Pharmaceutical Corp., Baltimore, MD 21224.

Rats were trained to criterion in a two-choice, discrete trial auditory/visual discrimination task. Responses on the right lever were reinforced in the presence of a bright light and responses on the left lever were reinforced in the presence of a tone. 24 hours later, and daily thereafter, 1/2 of the rats were injected i.p. with 1.7 mg/kg baclofen, a $GABA_B$ agonist. The other 1/2 received an equal volume of saline. 30 minutes later, rats were trained on a second problem with two new stimuli but with the auditory/visual dimensions constant (i.e., dim light-right, clicker-left). The third problem reversed this dimension/response relationship (tone-right, dim-left). Baclofen-treated rats required significantly more responses than saline controls to reach criterion for the second problem, but required significantly fewer responses to criterion in the third problem. Baclofen did not affect the probability of a response in either problem.

A Baclofen-induced retrieval impairment could contribute both to the slow learning in Problem 2 (positive transfer task) and to the relatively rapid learning of Problem 3 (negative transfer task). Alternatively, baclofen could simply impair learning. Although both groups met criterion in Problem 2, poorer learning of Problem 2 could decrease negative transfer to Problem 3, and hence increase the rate of acquisition. These hypotheses are being tested by drugging only on Problem 3. The learning deficit hypothesis predicts slower acquisition for Problem 3, while the retrieval deficit hypothesis predicts that baclofen before Problem 3 should lead to more rapid acquisition.

465.13

Accumulation of glutamate, aspartate and GABA in the hippocampus of gerbil after transient cerebral ischemia. G. Delbarre, B. Delbarre, F. Calinon*, C. Loirel* and A. Ferger*, Faculté de Médecine,37032, Tours, FRANCE.



To study memory of the gerbils, Cheal (Cheal, M.L., J. Biol. Psycho., 20 : 26, 1978) had developed tests to measure investigation of novel stimuli. In this test, occlusion of left carotid during 10 min induced a lost of memory. Glutamate and aspartate had been implicated in cerebral ischemia. In the hippocampus, 35 min and 90 min after ligation, amino acids (Aspartate, glutamate and GABA) had been measured by HPLC method after left carotid ligation during 30 min. Aspartate,glutamate and GABA were significantly increased 35 min and 90 min after ligation. The results indicated the necessity to select gerbil by occular fundus (Delbarre, G., Stroke, 19 : 126, 1988) 90 min after ligation to study the modifications of amino acids in the brain.

465.14

PROTECTIVE EFFECTS OF DM-9384, A PYRROLIDONE DERIVATIVE, ON CYCLOHEXIMIDE-INDUCED DECREASE OF GABA AND ACETYLCHOLINE RECEPTORS. T. Kameyama*, T. Nabeshima, K. Tohyama* and T. Shiotani* (SPON: K. Kamata). Dept. of Chem. Pharmacol., Fac. Pharmac. Sci., Meijo., Univ., Nagoya 468, JAPAN.

We have reported that N-(2,6-dimethyl-phenyl)-2-(2-oxo-1-pyrrolidyl) acetamide (DM-9384) ameliorates GABA antagonist- and acetylcholine (ACh) antagonist-induced amnesia (Xth Int. Congr. Pharmacol., Sydney, Abstract P301, 1987; Psychopharmacology, 96: Suppl. 226, 1988). Cycloheximide (CXM)-induced amnesia is also improved by DM-9384 and this effect is attenuated by the treatment with bicuculline and picrotoxin. CXM-induced amnesia is known to be ameliorated by physostigmine, muscimol and baclofen (Nabeshima et al., Pharmacol. Biochem. Behav. 31: 405, 1988). These results indicate that DM-9384 affects the GABA and AChergic neuronal systems and improves amnesia. In the present experiments, we attempted to investigate whether DM-9384 protects the CXM-induced decrease in the number of binding sites for GABA and ACh. Mice of the ddY strain were used for the experiments. Synaptic membrane (P_2B fraction) was prepared from mouse brain 24 hr after the CXM (60 mg/kg, S.C.) treatment. DM-9384 (5 mg/kg, P.O.) was administered 15 min before CXM; [^{3}H] muscimol and [^{3}H] QNB were used as the ligands. Results were as follows: CXM significantly decreased Bmax of [^{3}H] muscimol binding sites in the synaptic membrane; DM-9384 protected $GABA_A$ receptors from the effect of CXM; CXM significantly decreased [^{3}H] QNB binding to muscarinic receptors; DM-9384 and muscimol protected QNB binding sites from the effect of CXM; DM-9384 displaced [^{3}H] muscimol binding to the membrane, but not [^{3}H] QNB binding. These results suggest that DM-9384 protects the CXM-induced decrease of GABA and ACh receptors by binding to $GABA_A$ receptor-like binding sites and increasing protein synthesis.

BEHAVIORAL PHARMACOLOGY: OTHER

466.1

CHRONIC ANTIDEPRESSANT TREATMENT EFFECTS ON CONFLICT BEHAVIOR IN THE RAT. T.J. Hill*, C. Becker*, D.J. Fontana* and R.L. Commissaris. College of Pharmacy, Wayne State University, Detroit, MI 48202.

Antidepressant agents vary in their efficacy in the management of panic disorder in man. The present studies examined the effects of chronic post-test treatment with several antidepressants on behavior in the Conditioned Suppression of Drinking (CSD) conflict paradigm. The agents tested were the TCA desipramine (DMI; good anti-panic efficacy), buproprion (BUP; no efficacy) and trazodone (TRAZ; weak efficacy). Acute treatment (IP; 10-min pre-test) with these agents did not exhibit anti-conflict effects at any dose examined. As expected, chronic DMI treatment (5 mg/kg, IP, BID for 5 weeks) resulted in a time-dependent anti-conflict with a latency to onset of approximately 3 weeks. In contrast, chronic BUP treatment (up to 10 mg/kg, IP, BID over 8 weeks) did not affect CSD behavior. Finally, chronic TRAZ treatment (up to 40 mg/kg, IP, BID over 12 weeks) resulted in only a weak anti-conflict effect. These data indicate that not all antidepressant agents exhibit anti-conflict effects when administered chronically. Moreover, the efficacy of these antidepressants to increase punished responding when administered chronically correlates well with their efficacy as anti-panic agents in man. (MH#42501; protocol conforms to NIH guide).

466.2

BEHAVIORAL AND NEUROENDOCRINE CORRELATES OF I.C.V. CORTICOTROPIN RELEASING HORMONE ADMINISTRATION IN THE RHESUS MONKEY John R. Glowa, Clinical Neuroendocrinology Branch, NIMH, Bethesda, MD 20892.

Corticotropin releasing hormone (CRH, or factor) is now known to be released from hypothalamic nuclei in response to stressful events and initiate a pituitary-mediated adrenal glucocorticoid response. The current studies were designed to extend our knowledge of the behavioral consequences of the activation of this hypothalamic-pituitary-adrenal (HPA) response by directly administering CRH into the lateral ventricle of behaving monkeys. Four rhesus monkeys (macaca mulatta), previously trained to respond under a chain FI 5-min, FR 30 response schedule of banana pellet delivery until rates and patterns of responding were stable, were aseptically and stereotaxically implanted with a Heyer-Schulte peritoneal shunt in the left lateral ventricle. This catheter was then attached to a subcutaneously implanted vascular access port. After recovery there was no deficit in baseline rate of responding or effect of control injections (Elliott's B solution). Increasing concentrations (from 0.003 ng/kg-30 µg/kg) of CRH dose-dependently decreased responding in a manner that did not depend upon the schedule component in effect. When large rate-decreasing effects were obtained, monkeys did not eat pellets that were produced. Midazolam (0.03-3.0 mg/kg) increased responding and could block some of the rate-decreasing effects of CRH. These results are consistent with the notion that stress mediated release of CRH profoundly affects behavioral function, in particular food-maintained behaviors.

466.3

ALCOHOL- AND BARBITURATE-INDUCED HYPNOSIS FOLLOWING STRESS: CONTROLLABILITY AND DURATION ARE CRITICAL DETERMINANTS. R.C. Drugan, D.M. Scher*, V. Sarabanchong* & P. Holmes*. Dept. of Psychology, Brown University, Providence,RI 02912

Two experiments were conducted to examine the impact of controllability and duration of shock on both barbiturate- and alcohol-induced hypnosis in rats. In Experiment 1,rats were exposed to either 80 escapable or yoked-inescapable shocks. Immediately or 2 hours later, all rats were injected with a hypnotic dose of either sodium pentobarbital or ethanol and the duration of sleep-time was measured in comparison to naive controls. Both escape and yoked groups showed a significantly longer barbiturate-induced sleep-time when compared to naive controls. However, ethanol-induced hypnosis was differentially altered by the controllability of stress. Only the inescapably-shocked group demonstrated a longer ethanol-induced sleep-time in comparison to both escapably-shocked and naive controls,which did not differ from one another. In Experiment 2, rats were exposed to a more acute stress treatment, 20-5 second inescapable shocks, and were injected with either sodium pentobarbital or ethanol immediately or 2 hours post-stress. This acute stress exposure did NOT significantly alter the barbiturate or ethanol-induced hypnosis at either time point. These results suggest that controllability and duration of stress can markedly influence the hypnotic potency of 2 classes of central nervous system (CNS) depressants.

466.4

TWO PARAMETER CATEGORIZATION OF RAT LOCOMOTOR BEHAVIOR M.P. Paulus*, M.A. Geyer, and A.J. Mandell, Lab of Biol Dynamics and Theoret Med, UCSD Dept Psychiatry, CA 92093

An extensive analysis of rat locomotor data recorded in the Behavioral Pattern Monitor (BPM) was done to obtain a global measure of behavioral change induced by stimulant drugs. It has been previously described that rats exhibiting the same amount of locomotion may follow quite distinct patterns of locomotion suggesting that at least two global parameters are necessary to describe the changes induced by the drugs. The following drugs were evaluated: amphetamine, MDMA, lisuride, apomorphine, nicotine, and scopolamine. Briefly, the animals were exposed to the BPM for 60 min; their x,y-position, distinct behavioral events such as rearing or holepoking, and the time spent at each position was obtained. These data were used to calculate a measure of activity, which was assumed to be a function of the measuring instrument, consequently yielding a scaling relationship. A conventionally defined reference distance was used to define the number of movements per unit time. Additionally the complexity measure based, as previously described, on a "meaningful" partition of the (x,y,t,behavior)-space was calculated as a second parameter. The results were expressed in units of standard deviations from a population of controls pooled from several experiments. The transition scenarios observed point toward several behavioral categories that animals exhibit under the influence of stimulants. The functional similarity of the different drugs was evaluated as the distance of the induced changes in the two-dimensional parameter space. This approach provides a quantitative description of drug effects beyond a pure activity measure.

466.5

BEHAVIORAL ANALYSIS OF CONDITIONED LOCOMOTION IN RATS USING METHYLENEDIOXYMETHAMPHETAMINE (MDMA) AS THE TRAINING DRUG. L.H. Gold, G.F. Koob and M.A. Geyer, Research Institute of Scripps Clinic and UCSD Dept. of Psychiatry, La Jolla, CA 92037.

The behavioral profile of the conditioned locomotor response (CLR) produced by MDMA was examined using the Behavioral Pattern Monitor (BPM) system. The BPM provides detailed information regarding amount and qualitative patterning of locomotor activity and investigatory responses. Two injection-free baseline days were followed by 5 drug training days. Rats were divided into 3 groups, the conditioned group was injected with MDMA 5 mg/kg immediately prior to placement in the BPM chambers for a 60 min session and saline upon return to the home cages, the pseudoconditioned group was injected in the reverse sequence and the control group was injected with saline at both times. On the test days, all rats were injected with saline and tested for a CLR.

The unconditioned profile of MDMA consisted of increased crossovers (horizontal locomotion), decreased rearings and control levels of holepokes, whereas, the conditioned profile included increased crossovers concomitant with increased holepokes. The conditioned increase in locomotion was determined to last at least 3 days under extinction parameters. The rats were retested 19 days after their last extinction trial and compared with a naive group of rats. Relative to the control group and pseudoconditioned group, the conditioned group exhibited increased crossovers and the naive group exhibited increased crossovers, holepokes and rearings at this time. Furthermore, examination of the spatial representations of rats' locomotor paths on various days will contribute to the evaluation of the behavioral qualities of the CLR produced by MDMA.

(Supported by NIDA grants DA 05333, DA 04398 and DA 02925)

466.6

PHACLOFEN ANTAGONIZES THE ANTINOCICEPTIVE AND SEDATIVE EFFECTS OF (-)BACLOFEN. C.De Luca* and M. Massotti (SPON: M.Ferrari). Lab. Farmacologia, Istituto Superiore di Sanità, 00161-Roma, Italy.

Baclofen (BAC), a selective GABA-B agonist, induces myorelaxant, sedative and antinociceptive effects. In rats, (-)BAC delays the responses to tail-flick (ED_{50} 4.1 mg/kg ip) and hot-plate (ED_{50} 3.2 mg/kg ip) tests. These effects are associated with sedation and presence of a synchronized record in the electrocorticogram (EEG). At the dose of 7.5 mg/kg ip, the righting reflex is absent.

In mice, the antinociceptive effect of (±)BAC is counteracted by phaclofen (PHA) (12.5-50 µg icv), a phosphonic acid derivative of BAC (Giuliani,S., Eur.J.Pharm., 154: 225,1988).

In rats, pretreatment (1 min) with PHA icv (100 µg) but not systemically (50 mg/kg ip) counteracts the responses of (-)BAC: 1) a competitive antagonism is observed in tail-flick (ED_{50}, 6.9 mg/kg) and hot-plate (ED_{50}, 6.8 mg/kg) tests; 2) the EEG pattern is desynchronized; 3) the righting reflex is present.

These data indicate that GABA-B PHA-sensitive receptors mediate the sedative and antinociceptive effects of (-)BAC in rats.

466.7

THE EFFECTS OF SYSTEMICALLY ADMINISTERED DOPAMINERGIC AND BRAIN REGIONAL GABA-ERGIC AGONISTS AND ANTAGONISTS ON REACTION TIME RESPONSE PARAMETERS IN THE RAT. R.D. Mayfield*, P.K. Randall, W.W. Spirduso, and R.E. Wilcox, Institute for Neuroscience, Depts. of Pharmacol. and *Kinesiol., Univ. of Texas at Austin, Austin, TX 78712

Intraperitoneally administered dopamine (DA) agonists and antagonists as well as microinjections of muscimol (MUS; GABA agonist) and bicucculline (BIC; GABA antagonist) into the substantia nigra reticulata (SNr), a major striatal efferent target, were studied for their effects on different parameters of the reaction time (RT) response in the rat. Animals were shaped to release a lever in response to an auditory/visual stimulus in order to avoid mild footshock. The response parameters of primary interest are percent successful avoidance (% avoidance), the latency (speed) of the successful response, and response consistency. The % avoidance as well as the successful latencies were impaired by DA antagonists. Percent avoidance was decreased by approximately 60 and 50% with 100 µg/kg of SCH 23390 (SCH) and haloperidol (HAL), respectively. The response latencies were increased by about 30 (65 msec) and 40% (130 msec) in SCH and HAL treated animals, respectively. The selective D2 antagonist spiperone (1.0 and 10 µg/kg, i.p.) produced similar though less pronounced effects. Microinjections of BIC (10 and 50 ng/5 µl) into the SNr produced a similar pattern of disruption, decreasing % avoidance by about 47% and increasing the successful latencies by about 45% (≈130 msec) at the high dose. This pattern of disruptionwas not observed with the direct acting agonist apomorphine (i.p.) or with MUS microinjections (5 and 25 ng/5 µl). Apomorphine produced a decrease in % avoidance similar to that of SCH (≈46%) but the response latencies were not affected. Similarly, microinjections of MUS into the SNr decreased % avoidance (≈40%) but had little effect on the response latencies. Amphetamine had no effect on % avoidance while slightly decreasing response latencies at the doses used in these studies (0.1, 0.5, and 1.0 mg/kg). These results suggest that different parameters of the RT response are sensitive to systemically administered DA agonists and antagonists in a manner consistent with the effects of GABA agonists and antagonists in the SNr. (Supported by NS20827 and MH44799)

466.8

CHRONIC HINDLIMB FLEXION IN RAT IS SUPPRESSED BY 5-HT_2 AGONIST, DOI, AND RESTORED BY 5-HT_2 ANTAGONIST, KETANSERIN. M.F. Anderson*, D.J. Mokler and B.J. Winterson. Depts of Physiology and Pharmacology, University of New England College of Osteopathic Medicine, Biddeford, ME 04005

Electrical stimulation for 1h across a rat hindlimb induced an ipsilateral flexion. At 72h, flexion was $\bar{x}$=8.3g . Spinal section at the mid-thoracic level resulted in a significant increase (rebound) in flexion ($\bar{x}$=13.0g).

Previously, we reported that either depletion of central 5-HT stores or non-selective 5-HT antagonism eliminated rebound. Presently, (±)-1-(2,5,Dimethoxy-4-iodophenyl)-2-aminopropane (DOI) was administered after spinal section and the effect of cumulative doses assessed. Flexion averaged 5.7g at 72h and increased to 9.3g with spinal section. Between 3.0 and 10.0mg/kg, i.p., flexion was dramatically suppressed ($\bar{x}$=0.2g). Subsequent administration of ketanserin (3.0-10.0mg/kg,i.p.) resulted in a 91% recovery of flexion. Also, the selective 5-HT_{1B} agonist, (±)-1-(3-(Trifluoromethyl) phenyl)-piperazine (TFMPP), was administered in cumulative doses (0.1-10.0mg/kg, i.p.) prior to spinal section. No dose produced a change in flexion ($\bar{x}$=8.4g). However, the amount of rebound was significantly reduced in comparison to controls (2.2g vs 4.7g).

These results suggest that supraspinal inhibition is modulated by the combined effects of postsynaptic 5-HT_2 and presynaptic 5-HT_{1B} receptor stimulation.

(Supported by AOA Grant #88-11-290)

466.9

TEMPORAL COMPARISON BETWEEN FENFLURAMINE AND ITS ACTIVE METABOLITE NORFENFLURAMINE. D.M. Lovano-McBurney* and M.D. Schechter, Dept. Pharmacol. Northeastern Ohio Univ. Coll. of Med., Rootstown, OH 44272

Male rats were trained to discriminate intraperitoneally administered 2.0 mg/kg fenfluramine (FEN) from its vehicle using a two-lever, food-motivated operant discrimination task. Once trained, the rats showed a dose-dependent decrease in discriminative performance on the FEN-correct lever when tested 20 min following decreasing doses of FEN (ED50 = 1.09 mg/kg). Administration of 2.0 mg/kg norfenfluramine (NF) 20 min prior to testing produced 94.4% responding on the FEN-correct lever. The 2.0 mg/kg dose of both FEN and NF was tested at post-administration times ranging from 5 min to 1440 min (24 hrs) and time-course data indicated that FEN has a peak effect from 20-240 min after administration. NF was observed to have a more rapid onset and a longer duration of action in the FEN-trained rats. These time-course results confirm previous work in this laboratory (Pharmacology Biochemistry & Behavior 31:305-311, 1988) using rats trained to discriminate NF and indicate a pharmacological similarity between NF and FEN. However, the difference in onset and offset of action suggests a possible difference between the parent drug and its metabolite.

This work was supported by NIDA grant No. 04181.

466.10

SELECTIVE BREEDING OF RATS FOR HIGH AND LOW MOTOR ACTIVITY IN A SWIM TEST: QUANTITATIVE AUTORADIOGRAPHIC ANALYSIS OF α_2 ADRENOCEPTORS IN THE LOCUS COERULEUS (LC). GB Kovachich[1,2], MA Cierpial[3], CE Aronson*[1], A Frazer[1,2] and JM Weiss[3]. Univ of PA[1] & Vet Admin Med Ctr[2], Phila PA, 19104; Duke Univ Med Ctr[3], Durham NC 27710.

Weiss and colleagues have initiated a breeding program to generate strains of rats which display either high or low motor activity (H & L, respectively) in a swim test. After four generations of selective breeding, H and L rats were subjected to: **1)** inescapable shock (3 hr randomly spaced shocks) followed by a 15 min swim test; **2)** only the swim test; or **3)** neither condition (controls). Values (mean ± SEM, n=5) are:

Strain	Treatment	Activity Score (seconds)	^{3}H-Idazoxan Binding (fmole/mg Protein)
H	control	-	1084 ± 11
H	swim	-86 ± 59	943 ± 31
H	shock+swim	-214 ± 49	961 ± 34
L	control	-	899 ± 21
L	swim	-508 ± 36	1022 ± 21
L	shock+swim	-544 ± 49	993 ± 29

In these two strains of rats, the baseline level of ^{3}H-idazoxan binding to α_2 adrenoceptors in the LC is significantly different. In each strain, either stressor (swim or shock plus swim) produced comparable changes in α_2 adrenoceptors; however, in H rats, ^{3}H-idazoxan binding decreased whereas it increased in L rats. **(Supported by the Vet. Adm. & USPHS grant MH40406.)**

466.11

SELECTIVE BREEDING OF RATS FOR HIGH AND LOW MOTOR ACTIVITY IN A SWIM TEST: BEHAVIORAL, ELECTROPHYSIOLOGICAL AND PHARMACOLOGICAL EFFECTS. M.A. Cierpial, P.E. Simson, L.A. Rankin*, M.D. Carpenter*, P.A. Scott* and J.M. Weiss. Dept. of Psychiatry, Duke Univ Med Ctr, Durham, NC 27710.

A selective breeding program was undertaken to generate populations of rats which display either high or low motor activity in a swim test. Eighty-two male and 42 female Sprague-Dawley rats were screened in a 15 minute swim test in which the total time spent struggling and floating was measured. The most active and least active males and females were then identified and bred. This screening and breeding process has been repeated for five generations producing rats that differ markedly in the swim test.

Fourth generation Highs and Lows were examined on a number of measures (see also accompanying abstract). Electrophysiologically, locus coeruleus (LC) neurons of Lows displayed higher spontaneous and evoked activity than those of Highs. This pattern of LC activity in Lows resembles that seen in normal animals following pharmacological blockade of α-2 receptors. Consistent with these electrophysiological results, subcutaneous injection of the α-2 blocker yohimbine decreased swim test struggling in Highs, while the anxiolytic diazepam, which has been shown to decrease LC responsiveness, increased swim test struggling in Lows. These results suggest that the behavioral differences observed between these rat lines are mediated through alterations in LC functioning.

466.12

CHRONIC CLONIDINE ADMINISTRATION AND CONFLICT BEHAVIOR IN THE RAT. D.J. Fontana*, D.M. Schefke* and R.L. Commissaris (SPON: W. Crossland). College of Pharmacy, Wayne State University, Detroit, MI 48202.

The effects of chronic clonidine administration on conflict behavior were investigated. In daily 10-min sessions, water-deprived rats were trained to drink from a tube that was occassionally electrified (0.25 mA); electrification was signalled by a tone. Prior to treatment, subjects accepted 30-40 shocks/session (punished responding) and consumed approximately 10-12 ml/session (unpunished responding). Chronic post-test clonidine administration (40 ug/kg, IP, twice daily for 18 weeks) resulted in a robust and time-dependent increase in punished responding (80-90 shocks/session) relative to controls. The responses to acute pre-test challenges with Ro15-1788 (no effect) and alprazolam (increase in punished responding) were similar in the two groups, suggesting no change in benzodiazepine receptor function. In contrast, the alpha-2 antagonist yohimbine exerted a greater anti-conflict effect in the subjects receiving chronic post-test clonidine. Finally, the anti-conflict effects of chronic clonidine were not prevented by the adrenergic neurotoxin DSP-4. These findings suggest that chronic clonidine treatment produces an anti-conflict effect through post-synaptic alpha-2 receptors. (MH #42501; conforms with NIH Guide).

466.13

REM SLEEP ENHANCEMENT: β-ADRENERGIC BLOCKADE OF THE PONTINE FTG BY TIMOLOL. A. Mamelak*, J. Quattrochi, M. Wolf*, and J.A. Hobson. Laboratory of Neurophysiology, Harvard Medical School, Boston, MA 02115

The β-adrenergic receptor blocker, timolol, was microinjected into the gigantocellular tegmental field (FTG) and its pharmacologic effect on rapid eye movement (REM) sleep studied in 3 unanesthetized adult male cats. Our protocol consisted of 4 baseline control polygraphic recordings for each cat (n=12) followed by 24 injection trials with timolol (4μg/250nl) at 6 pontine sites (2.5P, 1.6L, -3.7V). Results indicate a 300% increase in the amount of time associated with PGO wave activity ($p< 0.001$) compared to controls, and an enhancement of REM sleep behavior. Mean REM sleep latency (29.8± 5.3 min.) and PGO latency (12.8±4.5 min.) were significantly shortened ($p<0.01$) compared to controls (108.2± 48.5 and 70.7±41.0). The mean percent of recording time in REM sleep (14.7±1.4%) also was increased compared to controls (4.3±1.2%). These data, coupled with results from carbachol enhancement of REM sleep, suggest that blockade of adrenergic inputs to the FTG by timolol is an effective means of experimental REM sleep induction, and support the hypothesis that physiologic REM sleep may be produced from a combined aminergic withdrawal and cholinergic excitation at the level of the pontine brain stem. Supported by NIH grant MH-13923.

466.14

ALPHA-2-RECEPTORS AND VIGILANCE IN CATS: ANTAGONISM OF MEDETOMIDINE SEDATION BY ATIPAMEZOL. D. Stenberg* and T. Porkka-Heiskanen (SPON: G. Grant). Dept.of Physiology, Univ.of Helsinki, 00170 Helsinki, Finland.

In order to evaluate the specific alpha-2-receptor antagonist atipamezol as to its effect on vigilance, adult cats with implanted electrodes for polygraphy were tested in a double blind Latin square design. The standard clinical dose 100 ug/kg im. of the specific alpha-2-agonist medetomidine promptly induced stuporous sedation. Atipamezol, given 30 min later at 200,400 or 800 ug/kg im., reversed the sedation within 2 min, resulting in complete awareness of the animal. One hour after the largest dose of atipamezol, the physiological sleep-wake cycle had returned, whereas after the small dose, arousal with some motor excitation continued for 7 hours. Used alone, the preferred dose 400 ug/kg atipamezol im. allowed physiological sleep within 40±7 min, compared to 22±2 min after saline.

Atipamezol thus proves a most effective antagonist to sedation with alpha-2-agonist drugs, without disturbing excitatory effects. Specific alpha-2-receptor modulating drugs have evident clinical application.

466.15

MDL 27531 IS A SELECTIVE STRYCHNINE ANTAGONIST. F.P. Miller, J.M. Kane, D.L. Braun, H.J. Ketteler*' T.C. McCloskey and J.H. Kehne. Merrell Dow Research Institute, Cincinnati, OH 45215.

MDL 27531 [4H-1,2,4-triazole,4-methyl-3-(methylsulfonyl)-5-phenyl-] is a specific antagonist of strychnine-induced seizures in mice relative to its effects in other seizure tests. Thus, its ED50 against strychnine-induced seizures was 12.8 mg/kg ip, whereas against electroshock-, pentylenetetrazol-, and 3-mercaptopropionic acid-induced seizures, ED50 values were >50 mg/kg. CNS depressant effects are minimal, since the ED50 vs motor activity is >200 mg/kg, and the LD50 is >800 mg/kg ip. Many known muscle relaxants are moderately effective against strychnine-induced seizures in mice, but cause CNS depressant activity at effective doses.

This profile was generally reflected in a rat model in which MDL 27531 selectively attenuated the excitatory effect of a subconvulsant dose (0.9 mg/kg ip) of strychnine on acoustic or tactile startle reflexes. MDL 27531 did not depress baseline and did not antagonize the enhanced reflexes produced by rolipram (0.5 mg/kg ip) or d-amphetamine (4.0 mg/kg ip). In contrast, diazepam (5.0 mg/kg ip) or gamma-vinyl-GABA (1.9 g/kg ip) markedly depressed startle baseline but did not diminish strychnine excitation.

It is suggested that MDL 27531 may be a novel centrally-acting muscle relaxant with minimal CNS depressant activity.

467.1

L- AND D-BACLOFEN INDUCE DIFFERENT CARDIOVASCULAR RESPONSES IN THE RAT AFTER INTRATHECAL ADMINISTRATION. Y. Hong and J.L. Henry (SPON: S.N. Young) Depts of Physiology & Psychiatry, McGill Univ., Montreal, H3G 1Y6

Our previous results indicated that bicuculline-sensitive $GABA_A$ receptors in the rat spinal cord are involved in regulation of arterial pressure and heart rate (Soc. Neurosci. Abst. 14:190). The purpose of the present study was to examine the possible role of $GABA_B$ receptors by determining the effects of L- and D-baclofen given intrathecally at the T2 and T9 spinal levels. Anaesthetized (urethane, 2.5g/kg, i.p.) male Sprague Dawley rats (320-350g) were used. Intrathecal administration of L-baclofen at T2, in doses of 7 (n=6), 35 (n=7) and 70 (n=7) nmols in artificial CSF, induced a dose-dependent increase in arterial pressure without altering heart rate; the effect appeared within 30 s following administration, peaked at 2 min and decayed over the next 5 min. Injection i.v. of 70 nmol of L-baclofen had no effect on arterial pressure or heart rate. Intrathecal administration of D-baclofen at T2, in doses of 7 (n=7), 70 (n=10) and 700 (n=6) nmols, induced dose-dependent decreases in both arterial pressure and heart rate; responses began within 1-2 min, and persisted for 9-30 min, depending on the dose. Intrathecal administration of 70 nmols of L- and D-baclofen at T9 induced similar responses to those seen at T2. Responses to both forms of baclofen were blocked by pretreatment with (i) hexamethonium (10 mg/kg, i.v., n=7) and (ii) lidocaine (15 uL of a 1% solution, intrathecally, n=7). Control rats, given CSF only, exhibited no responses to injection. The results suggest that L- and D-baclofen-sensitive GABA receptors in the spinal cord are involved in regulation of spinal sympathetic output controlling arterial pressure and/or heart rate.

(Supported by a grant from the Quebec Heart Foundation to JLH)

467.2

ACTIVATION OF 'POSTSYNAPTIC' $GABA_B$-RECEPTORS IN THE NUCLEUS OF THE SOLITARY TRACT (NTS) INHIBITS BAROREFLEX BRADYCARDIA A. Florentino* and G. Kunos* (SPON: B. Tabakoff) Lab. Physiol. & Pharmacol. Studies, NIAAA, Bethesda, MD 20892

The effects of bilateral intra-NTS microinjections (50 nl/side) of GABAergic agents on arterial blood pressure (BP), heart rate (HR) and baroreflex bradycardia were tested in urethane-anesthetized rats. Baroreflex bradycardia was elicited by i.v. injection of graded doses of phenylephrine, and was quantified as the increase in pulse period in response to a unit increase in BP. Microinjection of the $GABA_A$ agonist, muscimol (90 pmol/side), the $GABA_A$ antagonist, bicuculline (10 pmol/side), or the $GABA_B$ antagonist, phaclofen (200 pmol/side) did not influence BP, HR or baroreflex bradycardia. Intra-NTS injection of the $GABA_B$ agonist, baclofen (20 pmol/side) caused a slow but sustained rise in BP and HR, and a marked reduction in baroreflex bradycardia. Simultaneous intra-NTS injection of phaclofen (200 pmol/side) failed to influence the effects of baclofen. The results indicate that stimulation of $GABA_B$ receptors in the NTS region cause pressor effects, probably by inhibiting the depressor baroreflex response. Phaclofen-insensitive $GABA_B$ receptors have been proposed to be 'postsynaptic' to GABAergic terminals (Kerr, D.I.B. et al., *Brain Res.* 405:150, 1987). Therefore, the above effects of baclofen may be due to inhibition of the release of a transmitter other than GABA, which directly mediates or tonically facilitates the depressor baroreflex response.

467.3

GABA ACTS ON GABA-B RECEPTORS IN NUCLEUS TRACTUS SOLITARIUS TO INCREASE BLOOD PRESSURE. J.C. Sved and A.F. Sved. *Department of Behavioral Neuroscience, University of Pittsburgh, Pittsburgh, PA 15260.*

Local injection of nipecotic acid (NIP), an inhibitor of GABA uptake, into the nucleus tractus solitarius (NTS) of chloralose-anesthetized rats increases arterial pressure (AP) presumably by potentiating the action of endogenously released GABA. Although agonists of the GABA-A receptor increase AP when administered into the NTS, the pressor response to NIP is not antagonized by drugs that block the GABA-A receptor. The present studies examined the effects of a newly described inhibitor of the GABA-B receptor, phaclofen (PHAC). PHAC (4 nmol in 100 nl artificial CSF) administered bilaterally into the NTS slightly decreased AP (-8±2 mmHg, n=6, $P<0.05$) while heart rate did not change. Higher doses were not tested due to the drug's limited solubility and lower doses did not significantly change AP. PHAC (4 nmol) reversed the pressor response to NIP (10 nmol) injected into the NTS. PHAC (0.1-4 nmol) administered into the NTS antagonized in a dose dependent manner the pressor effect of intra-NTS injections of baclofen (1-100 pmols), a specific GABA-B agonist. PHAC did not alter the pressor response to injection into the NTS of the selective GABA-A agonist muscimol. These results demonstrate the pressor response to NIP injected into the NTS is mediated via GABA-B receptors, suggesting that in the NTS endogenous GABA influences cardiovascular function predominantly through an action on GABA-B receptors.

467.4

MICROINJECTION OF MUSCIMOL INTO POSTERIOR HYPOTHALAMUS BLOCKS CARDIOVASCULAR RESPONSE TO STRESS IN RATS. J.A. DiMicco, M. Lisa, E. Marmo, J.H. Wible, Jr.. Depts. of Pharmacol. & Toxicol., and Med., Ind. Univ. Sch. of Med., Indianapolis, IN 46223, and Inst. of Pharmacol. & Toxicol., 1° Fac. Med. Chir., Univ. Napoli, Naples, Italy

Microinjection of GABA antagonists into the posterior hypothalamic nucleus (PH) produces cardiovascular and behavioral changes resembling those seen in stress. Here, we examined the effect of similarly injecting muscimol (MUS), a $GABA_A$ receptor agonist, on heart rate (HR) and blood pressure (BP) under resting and "stress" conditions in conscious instrumented rats. At sites in the PH where GABA blockade caused marked tachycardia under anesthesia, MUS (10ng/side) or saline (SAL; 250nl/side) was injected 5 min prior to a 20 min observation period of either RESTING (in home cage) or STRESS (restrained in a stream of air). Six rats were subjected to every treatment in random order at 2-3 day intervals.

	HR (beats/min)		BP (mmHg)	
	BASAL	+10 MIN	BASAL	+10 MIN
RESTING/SAL	378±10	+10±13	110±3	+4±2
RESTING/MUS	377±12	-28±5*	106±5	-6±3
STRESS/SAL	356±8	+130±14*	104±3	+14±3*
STRESS/MUS	362±10	+9±8	98±6	+2±3

* change from BASAL by paired t-test, $p<0.05$

These results suggest that stimulation of $GABA_A$ receptors in the PH blocks the HR response to experimental stress in rats. (Supported by USPHS grant NS 19883)

467.5

THE EFFECTS OF INTRACEREBROVENTRICULARLY INFUSED GABA IN SPONTANEOUSLY HYPERTENSIVE AND NORMOTENSIVE RATS. Kim A. Roberts, Joseph Harding and John W. Wright, Washington State University, Pullman, WA., 99164. Intracerebroventricular (icv) infusion of GABA at various doses was investigated in Spontaneously Hypertensive rats (SHRs), and Wistar-Kyoto (WKY), and Sprague Dawley (SD) normotensive rats. Previous investigations have reported that icv injections of GABA, or muscimol the potent GABA agonist, produced hypotension in rats (Perrson, 1980). In this experiment GABA at doses of 0, 100, 1,000 and 10,000 nmoles/2µl aCSF/min was infused for 5 min in alert rats. Results indicate significantly lowered mean arterial blood pressure (MABP) in members of all three strains at doses of 1,000 and 10,000 nmoles/min, with maximum decreases in MABP at approximately 10 min postinfusion. In the SHRs the maximum decrease in MABP for the 10,000 nmole/min dose, was 84 mmHg lasting 90 min; in the normotensive strains the maximum decrease in MABP was 34 mmHg and required 50 min for recovery. Our findings agree with earlier reports suggesting that icv injections of GABA significantly alter blood pressure (Unger et al., 1984); however, by utilizing three strains of rats and adminstering GABA at several doses we were able to demonstrate that icv infusions of GABA decreased MABP from baselevel in a dose-dependent fashion in members of all strains, with the SHRs being supersensitive to central infusions of GABA. Supported by NIH grants HL32063 and TW01112 and the American Heart Association.

467.6

CARDIORESPIRATORY EFFECTS CAUSED BY MICROINJECTION OF NMDA OR KAINIC ACID (KA) INTO THE POSTERIOR HYPOTHALAMUS (PH) IN ANESTHETIZED RATS. R.P. Soltis* and J.A. DiMicco (SPON: G. Nicol). Dept. of Pharmacol. & Toxicol., Ind. Univ. Sch. of Med., Indianapolis, IN 46202-5120.

Drugs which interfere with the inhibitory function of the neurotransmitter gamma aminobutyric acid (GABA) act within the PH to produce cardiovascular, behavioral and respiratory changes resembling those seen in stress in rats. This study examined the hypothesis that injection of excitatory amino acid receptor agonists into the PH would cause similar effects in urethane-anesthetized rats. NMDA or KA was microinjected in 50 nL of saline at the same site in the PH where injection of the GABA antagonist bicuculline methiodide 10 ng produced tachycardia (>90 beats/min). Below are mean data ± SEM for heart rate (HR; beats/min), mean arterial pressure (MAP; mmHg), and respiratory rate (RR; breaths/min) (n=4).

	DOSE	HR		MAP		RR	
	pmol	base	change	base	change	base	change
NMDA	6.8	351±19	+13±1	103±2	-3±1	103±5	+1±1
	20.4	371±16	+36±6	104±4	+5±2	112±3	+13±3
	68.0	375±15	+65±5	106±3	+7±1	105±4	+23±3
KA	4.7	380±8	+25±4	99±3	+4±1	115±20	+13±6
	14.1	381±12	+45±5	104±4	+7±1	107±9	+32±7
	47.0	384±21	+73±8	103±4	+13±2	113±2	+60±16

Our findings show that injection of either NMDA or KA into the PH of the rat produces dose-related cardiorespiratory stimulation. (Supported by USPHS NS 19883)

467.7

INHIBITION OF THE ARTERIAL BAROREFLEX BY A SPECIFIC N-METHYL-D-ASPARTATE RECEPTOR ANTAGONIST. G. Hajduczok and R.B. Felder. Dept. of Int. Med. and CV Center, Univ. of Iowa College of Medicine, Iowa City, IA 52242.

Recent studies have suggested that N-methyl-D-Aspartate (NMDA) receptors are involved in baroreflex regulation. We tested the effects of the specific non-competitive NMDA antagonist (+)-5-methyl- 10,11-dihydro-5H-dibenzo-[a,d] cyclohepten-5,10-imine maleate (MK-801) on baroreflex control of sympathetic nerve activity (SNA). Renal (RSNA, n=5) and cardiac (CSNA, n=4) sympathetic nerve activities were measured in 5 chloralose anesthetized cats before and after intravenous injection of MK-801 (0.5 mg/kg). The baroreflex was assessed during ramp increases in arterial pressure over the range of 60-200 mmHg. MK-801 administration did not change mean arterial pressure (MAP) but decreased both the maximum gain (Gmax) and the range (R) of inhibition of the MAP vs. SNA relation. (* significantly different from Control (C), p<0.01).

	Gmax (%/mmHg)		R (% Δ SNA)	
	C	60 min	C	60 min
RSNA	1.69±0.2	0.43±0.34*	67.7±7.5	8.6±2.1*
CSNA	1.21±0.3	0.13±0.1*	65.5±16	8.4±1.7*

Inhibition of the baroreflex was evident at 10 min and remained suppressed at 3 hours following MK-801 administration. These data are consistent with the view that central excitatory amino acid pathways acting on NMDA receptors mediate the arterial baroreflex.

467.8

MECHANISMS OF THE CENTRAL CARDIOVASCULAR EFFECTS OF ADENOSINE: INTERACTION WITH BRAIN L-GLUTAMATE. R. Mosqueda-Garcia, M. Appalsamy*, and D. Robertson. Dept. of Pharmacology, Vanderbilt University, Nashville, TN 37232.

We have recently reported that adenosine (ADO) has potent depressor effects when microinjected in the brainstem. In the present study we elucidated some of the neuronal connections involved in the mechanism of action of ADO and tested if the integrity of glutamate neurons in the brainstem is needed for ADO effects.

Sprague-Dawley rats anesthetized with urethane were cannulated for direct recording of BP and HR. Microinjections into the area postrema (AP), nucleus of the solitary tract (NTS) or rostroventrolateral medulla (RVLM) of ADO (2.3 nmol), the adenosine antagonist caffeine (30 mg/kg iv) or glutamate antagonist, kynurenic acid (KYN, 33 nmol), or DL-2-amino-5-phosphono-valeric acid (APV, 0.6 nmol) were done using stereotaxic coordinates and pharmacological responses. Sympathetic nerve activity (SNA) was recorded from the left renal nerve.

Intra-NTS ADO decreased BP by -22±2 mmHg, HR by -24±4 b/m and SNA by -43±8%, n=6. These effects were inhibited by either previous administration of ADO or glutamate antagonists. More potent effects were seen after intra-AP microinjection of ADO (-27±3 mmHg; -28±9 b/m; -53±7% for BP, HR and SNA, respectively, n=7). Direct microinjection of ADO was ineffective in the RVLM, but all glutamate antagonists inhibited the cardiovascular effects of intra-AP or intra-NTS ADO (-4±2 mmHg; -11±4 b/m after APV in the RVLM, n=8).

These results suggest that the function of glutamate neurons is important for the cardiovascular effects of ADO. These glutamate effects are probably mediated by an NMDA-excitatory amino acid type of receptor.

467.9

BLOCKADE OF NON-N-METHYL-D-ASPARTIC ACID (NMDA) RECEPTORS IN THE NUCLEUS OF THE TRACTUS SOLITARIUS (NTS) ELIMINATES AORTIC BAROREFLEXES. Christina Leone* and Frank J. Gordon, Dept. of Pharmacol., Emory Univ. Sch.of Med., Atlanta, GA 30322.

The NTS is the central termination site of peripheral baroreceptor afferents. This laboratory has shown that blockade of NMDA, kainate (KA) and quisqualate (QA) receptors with kynurenic acid in the rat NTS is sufficient to abolish decreases in blood pressure and heart rate evoked by electrical stimulation of the aortic nerve (Faseb J. 3: A858, 1989). The purpose of the present studies was to investigate a potential role for non-NMDA receptors in the NTS in the mediation of aortic baroreceptor reflexes. Baroreceptor reflexes were elicited by graded electrical stimulation of the aortic nerve to produce depressor responses of -10±1, -20±1 and -36±2 mmHg. Ten min. following bilateral microinjections (50 nl) into the medial NTS of the non-NMDA receptor antagonist dinitroquinoxalinedione (DNQX) (100 pmol), depressor responses elicited by aortic nerve stimulation were reduced to -2±1, -5±1 and -7±3 mmHg, respectively (p<0.05). Injections into the NTS of vehicle alone (1.5% dimethylsulfoxide in artificial cerebrospinal flluid) had no effect on baroreflex responses. Aortic baroreflexes were also not inhibited by microinjections of DNQX into medullary sites surrounding the NTS. Depressor responses were also measured after direct injections into the NTS of ED50 doses of the selective EAA receptor agonists KA and the QA analog, α-amino-methylisoxazole-proprionic acid (AMPA). DNQX reduced depressor responses produced by KA from -20±3 to -8±4 mmHg, and those of AMPA from -27±5 to -4±3 mmHg. In contrast, DNQX did not reduce depressor responses produced by ED50 NMDA (-15±3 mmHg before and -19±6 mmHg after DNQX). These results indicate that synaptic activaton of non-NMDA receptors in the NTS participates in the medation of aortic baroreceptor reflexes. Supported by PMAF predoctoral fellowship to C.L. and NIH-HL36907 to F.J.G.

467.10

CONNECTIONS OF IMMUNOCYTOCHEMICALLY IDENTIFIABLE GLUTAMATE-SENSITIVE AREA POSTREMA NEURONS IN SHR. D.K. Hartle and C.F. Phelix. Cardiovascular Laboratories, UGA, College of Pharmacy, Athens, GA 30602.

Populations of monoaminergic neurons have been found in the rat area postrema (AP). Some of these neurons are sensitive to the excitotoxic effect of systemically administered glutamate (G). The AP has cardiovascular functions that are involved in hypertension. Destruction of G-sensitive AP neurons has a profound effect on chronic hypertension in adult SHR. The present objectives were to study the projections and innervation of G-sensitive AP neurons. LM and EM examinations of immunocytochemically stained APs from SHRs (G-treated and control) were performed to investigate the innervation of TH-, DBH- and 5HT-containing AP neurons. DBH-, 5HT, NPY-, GABA- and glutamate-containing axon terminals overlap regions with degenerating monoaminergic neurons. Ultrastructural signs of degeneration included chromatin clumping, cistern formation of ER, destruction of mitochondria and vacuolization of cytoplasm. Routine and immuno- histological methods revealed that the AP is the only medullary site of neuronal degeneration with this MSG treatment. Degenerating axons were observed primarily in the mNTS, ROb, RVLM and SpNV. These connections help explain glutamate's chronic depressor effect in SHR. NIH-HLBI 10-21-RR262-215.

467.11

AUTORADIOGRAPHIC LOCALIZATION OF α_1-ADRENOCEPTOR BINDING SITES IN THE INTERMEDIOLATERAL CELL COLUMN OF THE CAT. S.A. MARKS,* M.R. DASHWOOD,* M.P.GILBEY AND A.G. RAMAGE*,[1] Department of Physiology and [1]Academic Department of Pharmacology, Royal Free Hospital School of Medicine, Rowland Hill Street, Hampstead, London NW3 2PF, England

A previous study from this laboratory failed to show any significant α_1-adrenoceptor binding in the intermediolateral cell column (IML) of the cat spinal cord (Dashwood, M.R. et al, Neuroscience, 15: 537- 551, 1985). However, recent observations (Yoshimura, M. et al, Brain Research, 414: 138-142, 1987) have shown an α_1-adrenoceptor mediated excitation of sympathetic preganglionic neurones in vitro. We therefore re-examined the distribution of α_1-adrenoceptor binding sites in the IML of the cat.

Experiments were carried out as described previously (Dashwood et al) using [^{3}H]prazosin as the α_1-adrenoceptor ligand, however, sections were exposed to hyperfilm for twelve weeks. The degree of binding to non-specific sites was established by incubating in the presence of excess phentolamine (10μM). Prazosin showed a marked binding in the IML at thoracic and lumbar levels. The difference between the previous results and those of the present study may be explained by the longer exposure times to the hyperfilm in the present case.

Supported by grants from The British Heart Foundation and the MRC.

467.12

CLONIDINE'S ANTIHYPERTENSIVE EFFECT IS NOT ASSOCIATED WITH ENHANCED BAROREFLEX-MEDIATED BRADYCARDIA. L. Watkins* and W. Maixner (SPON: B. Whitsel). Dept. of Pharmacology, U. of North Carolina, Chapel Hill, NC 27514

The effect of clonidine (1, 10, 25 ug/kg) on reflex bradycardia to infused and bolus phenylephrine was measured in pentobarbital-anesthetized (PA) and conscious (C) rats. In PA rats, clonidine (1 ug/kg) decreased baroreflex gain (bpm/mmHg) from 0.88±0.27 to 0.35±0.17 and from 0.65±0.25 to 0.34±0.11 for infused and bolus phenylephrine respectively. In C rats baroreflex gain was two to three times higher than in PA rats and did not change following clonidine. In spite of different effects on baroreflex gain, the hypotensive effect of clonidine was similar in PA and C rats. Separate experiments examined the effect of sympathetic (1 mg/kg atenolol) or parasympathetic (1 mg/kg methyl atropine) blockade on reflex bradycardia with and without clonidine. Both sympathetic and parasympathetic blockade decreased gain in PA and C rats. Clonidine reversed the atenolol-mediated inhibition of baroreflex gain in PA rats. These results suggest that clonidine's hypotensive effect is not secondary to enhancement of baroreflex gain. Supported by DE08013 (W.M.).

467.13

THERMAL AND BLOOD PRESSURE (BP) EFFECTS OF CLONIDINE MICRO-INJECTED INTO THE PARAVENTRICULAR HYPOTHALAMIC NUCLEUS (PVN) IN CONSCIOUS ONE KIDNEY GOLDBLATT HYPERTENSIVE RATS J. R. Wilson, S. Bhatnagar , and G. Kirouac*, Dept. Psych., U. of Manitoba, Winnipeg, Manitoba, Canada, R3T 2N2

The one-kidney Goldblatt (1K-GB) model of renovascular hypertension alters thermal tolerance, and central catecholaminergic processes, including those of the PVN. The PVN contains a large number of α_2 receptors and projects to preganglionic cell bodies controlling thermogenesis and cardiovascular function. We, therefore, studied the effects of stimulation of PVN α_2 receptors with clonidine (Clon), an α_2 agonist, on BP, rectal temperature (Tr), and O_2 consumption (VO_2), in conscious 1K-GB (n=26) and normotensive (NT; n=27) rats. Animals were implanted with cannulae in the PVN or non-PVN areas and fitted in indwelling aortic and jugular catheters. Over a 3-day testing period, they received 0, 1.0, 20.0 nmol (.5 uL) of Clon in the PVN or non-PVN areas, preceded by either iv saline or iv rauwolscine (Rau), an α_2 antagonist. Clon produced mild dose-dependent decreases in Tr and VO_2, in NT rats when injected into either the PVN or non-PVN areas, and Rau attenuated the decreases produced by 20 nmol Clon. Conversely, in 1K-GB rats, Clon produced a marked hypothermia only when injected into the PVN, and Rau potentiated the hypothermic effects of 20 nmol Clon. The increased thermal responsiveness of 1K-GB rats to PVN Clon is consistent with notions of plasticity of adrenoreceptor function in hypertension.

467.14

REGIONAL AND SUBCELLULAR DISTRIBUTION OF CLONIDINE-DISPLACING SUBSTANCE IN BRAIN. M.P. Meeley, P. Emsberger, L.K. Char*, M. Noponen* & D.J. Reis. Div. Neurobiology, Cornell Univ. Med. Coll., New York, NY 10021.

Clonidine-displacing substance (CDS) is a low-MW substance in brain defined by displacement of ^{3}H-*p*-aminoclonidine binding to brain membranes and clonidine-specific antibodies (1 Unit (U) = 50% displacement), and by contraction of rat gastric fundus (GF). We sought to determine whether CDS is (a) differentially distributed among brain regions, and (b) contained in subcellular fractions enriched with synaptosomes. Distribution of CDS was determined in methanol extracts of 8 dissected regions of fresh bovine brain. CDS was heterogeneously distributed. Radioreceptor assay indicated that highest levels were in hypothalamus (HYP) > dorsomedial (DMM) and ventrolateral (VLM) medulla $\geq$ midbrain, frontal cortex, striatum (4-26 U/g wet wt); cerebellar vermis and corpus callosum gave nonsignificant values (<2U/g). Radioimmunoassay showed CDS-like activity in striatum > HYP, VLM > DMM, as well as midbrain > frontal cortex. Specific biological activity was confirmed by contraction of GF only for cardiovascular areas, i.e., HYP, VLM and DMM. Subcellular distribution of CDS, specifically within nerve terminals, was also determined. A P_2 fraction of brain was osmotically shocked, and the soluble fraction purified as described for whole brain homogenates (Meeley et al., *Neurosci Lett* 84:84, 1988). CDS was present in P_2 fractions, as measured by radioreceptor assay (1 and 3U/g brain for P_2 and homogenate, respectively) and GF bioassay (1U elicited 38±4% standard maximal contraction, compared to 41±5% for homogenate.) We conclude that CDS, defined by three independent criteria, is heterogeneously distributed in brain, and released from a synaptosome-enriched fraction of bovine brain by osmotic shock. CDS may be a novel neurotransmitter or modulator which participates in central regulation of arterial pressure.

467.15

SEPARATE MECHANISMS BY WHICH CLONIDINE DEPRESSES INTRASPINAL AND GANGLIONIC SYMPATHETIC TRANSMISSION. Donald N. Franz, Scott C. Steffensen*, and Lewis C. Miner*, Dept. of Pharmacology, University of Utah School of Medicine, Salt Lake City, UT 84132

Previous studies indicate that clonidine (5-50 ug/kg) depresses sympathetic preganglionic neurons (SPGNs) by activating alpha-2 receptors that are negatively coupled to adenylate cyclase (J. Auton. Nerv. Syst. 19:199-209, 1987). The excitatory role of cyclic AMP is supported by marked increases in intraspinal transmission to SPGNs following microinjection of 1-2 ug of cyclic AMP analogs, forskolin, or RO 20-1724 into the SPGN neuropil. Clonidine (5-50 ug/kg) also produced marked, dose-dependent depression of transmission through isolated stellate ganglia in spinal cats. The depressant effects of clonidine at spinal and ganglionic sites were additive. Clonidine did not affect postganglionic discharges from direct stimulation of ganglia blocked by mecamylamine, indicating that clonidine acts presynaptically to depress release of acetylcholine from preganglionic terminals. In contrast to intraspinal transmission, ganglionic transmission was not affected by i.v. RO 20-1724 or microinjection of cyclic AMP analogs or forskolin into the ganglia. Cyclic AMP does not appear to be involved in ganglionic transmission, either pre- or postsynaptically, but does appear to increase excitability of SPGN perikarya.

467.16

ALPHA-2 ADRENOCEPTOR MEDIATED MODULATION OF NPY RESPONSE DURING HEMORRHAGIC HYPOTENSION IN ANESTHETIZED DOGS. R. Briand,* N. Yamaguchi,* J. Gagné,* R. Nadeau* and J. de Champlain. Fac. of Pharmacy, Fac. of Medecine (Physiol), Univ. of Montreal. Sacré-Coeur Hosp. Res. Center, Montreal, Québec, Canada H4J-3J7.

The acute effects of oxymetazoline (OXY:2 ug/kg) and idazoxan (IDZ:300 ug/kg) were evaluated on neuropeptide-Y (NPY) and catecholamine (CA: epinephrine and norepinephrine (NE)) release during a hemorrhage (H) of 15 mL/kg in pentobarbital anesthetized dogs. Plasma concentrations of NPY and CA were determined in aortic (AO) and portal venous (PV) blood during H. In control dogs (n=7), H increased significantly both AO-CA and AO-NPY. Changes in PV-NE levels were associated with a significant increase in PV-NPY. Following alpha-2 blockade with IDZ (n=7), PV-NE, PV-NPY as well as AO-NE and AO-NPY concentrations during H were significantly higher in the presence of IDZ. The administration of the alpha-2 agonist OXY (n=7) abolished the increase of NE and NPY observed in PV and AO blood during H. The present study demonstrates that NPY is co-released with neuronal NE from peripheral sympathetic nervous fibers during H induced hypotension. These results also indicate that, in peripheral sympathetic nerves, both NPY and NE release during H are concomitantly modulated by the presynaptic alpha-2 adrenoceptor mediated inhibitory mechanism.

Supported by CDA, CHF and MRC

467.17

PROJECTIONS TO THE NUCLEUS OF THE SOLITARY TRACT FROM THE A1 NORADRENERGIC CELL GROUP. S. Roder* and J. Ciriello (SPON: F. Tepperman), Dept. of Physiology, Univ. of Western Ontario, London, Canada, N6A 5C1.

A map of A1 noradrenergic projections to the nucleus of the solitary tract (NTS) in the cat was obtained using the anterograde tracer Phaseolus vulgaris leucoagglutinin (PHA-L) and the retrograde tracer horseradish peroxidase (HRP). In the first study, PHA-L was iontophoresed into the region of the A1 cell group, and transverse sections of the brainstem were processed immunohistochemically for the demonstration of either PHA-L or tyrosine hydroxylase containing fibers and terminals. PHA-L labelled cells within the injection site were observed in the A1 cell group. Labelled fibers and presumptive terminal boutons were observed primarily in the ipsilateral commissural (Com) and medial nuclei of NTS. In addition, a light projection was observed to the ipsilateral intermediate and lateral nuclei of NTS. In the second study, HRP was microinjected into the Com and transverse brainstem sections were processed using the TMB method. HRP retrogradely labelled cells were seen in the region of the A1 noradrenergic cell group of the caudal VLM, bilaterally with ipsilateral predominance. These data show that neurons in the A1 noradrenergic cell group project to regions of NTS that receive cardiovascular afferent inputs and suggest that VLM may influence neurons in NTS involved in cardiovascular regulation. (Supported by MRC of Canada).

467.18

EFFECTS OF CENTRAL ADMINISTRATION OF 8-OH-DPAT ON AUTONOMIC OUTLOW IN ANESTHETISED CATS. A.G. Ramage*, S.L. Shepheard* & D. Jordan. Departments of Pharmacology & Physiology, Royal Free Hospital Medical School, Rowland Hill Street, LONDON NW3 2PF, England.

5-HT_{1A} agonists (e.g. 8-OH-DPAT) given i.v. evoke a centrally mediated vagal bradycardia and sympathoinhibition (Ramage,A.G. & Fozard,J.R., Eur.J.Pharmac., 138:179, 1987). The present experiments were designed to examine the sites at which these effects are evoked.

Experiments were perfomed on cats anesthetised with α-chloralose (70 mg/kg) & sodium pentobarbitone (18 mg/kg). They were paralysed (vecuronium bromide 200 μg/kg) and artificially ventilated. Microinjection of 8-OH-DPAT into the IVth ventricle (cumulative doses 2.5-40 nmol/kg in 20 μL) caused dose-related falls in blood pressure and heart rate which reached maxima of 54±8mm Hg (mean±s.e.m) and 62 ±13 beats/min. These were associated with reductions in inferior cardiac, renal and splanchnic nerve activities which reached maxima of (63±12%), (93±5%) and (92±3%) respectively at the highest dose. Gastric motility was also increased but phrenic nerve activity, tracheal pressure and femoral arterial conductance were unaffected. Following application of atropine methonitrate (0.1 mg/kg) heart rate then increased by 51±17 beats/min.

The results confirm that 8-OH-DPAT evokes hypotension and bradycardia mediated by sympathoinhibition and increased vagal tone, and indicate that these effects are mediated by sites accessible from the IVth ventricle.

This work was supported by the Wellcome Trust.

467.19

POWER SPECTRAL ANALYSIS OF HEART RATE VARIABILITY IN PROPRANOLOL TREATED RATS. W.E. Rote*, and J.D. Connor. Dept. of Pharmacology, Col. Medicine, Penn State Univ., Hershey, PA, 17033.

Power Spectral Analysis (PSA) of heart rate variability (HRV) detects changes in autonomic tone in larger mammals. We studied two groups of methoxyflurane anesthetized rats (male, S-D, wt=255-455g); saline infused (S, n=6) and propranolol infused (P, 4mg/kg/hr, n=6) to determine the utility of PSA. Pre-infusion heart rate (HR) and power spectral densities in P and S groups were S=376.5±10.4 SE and P=351.7±9.4. During 1h S infusion, mean integrated HR was 364.2±7.6. while P decreased HR to 288.9±11.8. Power spectral analysis of HRV during S infusion showed a slight decrease in both the low and high frequency power and an increase in the mid frequency ranges (LOW=3.1±6.0; 0.01-0.1Hz,MID=+3.8±2.9; 0.1-0.2Hz,HIGH=+0.3±1.2; 0.2-0.3Hz). The P infusion caused a large decrease in power spectral density at the low frequency range (LOW=-20.6±3.7) and an increase in the mid and high frequencies (MID=+9.8±2.2; HIGH=+11.4±2.1). This indicates reduced sympathetic activity. Baseline data of S and P groups were similar ($p>0.05$) and only P altered the baseline spectra ($p<0.05$, t test). Propranolol, at beta blocking doses, decreases HR and HRV in the region of the power spectrum related to sympathetic activity. Thus, applying PSA of HRV in rats yields predictable results as in larger mammals with slower heart and respiratory rates.

CARDIOVASCULAR REGULATION VI

468.1

DISCHARGE DEPENDENCIES OF PAG NEURONS TO THE CARDIAC CYCLE DURING SLEEP-WAKING STATES. H. Ni*, J. Zhang, and R.M. Harper (SPON: S. Eiduson). Dept. of Anatomy & Cell Biology & Brain Res. Inst., UCLA, Los Angeles, CA 90024.

The periaqueductal gray region (PAG) has been shown by stimulation and lesion evidence to be related to cardiovascular activity. The PAG has a direct projection to pressor cells in the subretrofacial nucleus and projects to the ipsilateral medial solitary nucleus. The PAG also receives projections from rostral limbic structures related to affective control. Through these connections, PAG neurons could modify patterning of the cardiac cycle, especially that associated with the defense reaction. To determine whether PAG neuronal discharge and cardiac cycle are sleep state-related, we examined neuronal discharge timing relationships with the ECG and the correlation of tonic neuronal rate and heart rate during different states. Of 68 cells recorded extracellularly from the PAG in five undrugged, freely moving cats, 50 (74%) showed a phasic discharge timing relationship and/or a tonic discharge correlation with the cardiac cycle. Forty-eight (96%) of these cells were state dependent. The greatest number of phasic relationships were during quiet sleep (14/24), followed by waking (12/24), and then by rapid eye movement (REM) sleep (9/24). Tonic discharge relationships were observed most frequently in REM sleep (30/41), less often in waking (10/41), and even less often in quiet sleep (8/41). These results suggest that the PAG may be an important region in mediating state-related cardiac patterning. Supported by HL22418.

468.2

ACTIVATION OF RENAL SYMPATHETIC NERVE ACTIVITY DURING STATIC EXERCISE IN CONSCIOUS CATS. K. Matsukawa*, L.B. Wilson*, P.T. Wall and J.H. Mitchell* (SPON: W.J. Gonyea) Moss Heart Center, UT-Southwestern Dallas, TX 75235-9034.

We directly measured renal sympathetic nerve activity (RNA) during static exercise in awake cats. After the cats were trained to perform bar-press movements for 1 min using a forelimb, they were instrumented for recording RNA, arterial pressure (AP) and heart rate (HR). The exercise experiments were conducted 3-10 days after surgery. RNA, AP, and HR increased during static exercise. The increase in RNA had both an initial and a late component. The initial increase in RNA occurred simultaneously with the onset of exercise and the late increase in RNA was progressively developed 20-30 sec after the onset of exercise. AP increased in parallel with the renal nerve response. To examine any influence of the arterial baroreflex on the RNA responses to static exercise, we allowed the cats to perform static exercise when AP was changed by infusion of norepinephrine or nitroprusside. The increase in RNA in response to static exercise was almost abolished when resting AP was increased; whereas, it was enhanced when resting AP was decreased. Thus, we suggest that the abrupt increase in RNA during static exercise is evoked by central activation of the sympathetic nervous system and that the arterial baroreflex significantly influences RNA during exercise.

468.3

FOREBRAIN LIMBIC-LOCOMOTOR AREAS MAY INFLUENCE HINDLIMB VASCULAR RESISTANCE IN DOGS. P.T. Wall, G.A. Ordway* and J.H. Mitchell* Moss Heart Center, UT-Southwestern, Dallas, TX 75235-9034.

The nucleus accumbens and substantia inominata may be forebrain locomotor targets of the limbic system. We investigated the possible involvement of these forebrain areas in the neurally mediated cardiovascular responses associated with locomotion. Using chloralose-anesthetized dogs, we measured or calculated mean arterial pressure, heart rate, femoral arterial blood flow, and femoral arterial resistance (hindlimb vascular resistance) before and during stimulation (240 μV, 80 Hz, 1 msec) of the posterior medial nucleus accumbens or substantia inominata. Stimulation of the posterior medial nucleus accumbens or substantia inominata decreased femoral arterial resistance to 48% of pre-stimulation, while heart rate increased (+18 bpm) and mean arterial pressure fell (-10 mmHg); however, no motor activity was observed. By contrast, stimulation of subthalamic locomotor region or mesencephalic locomotor region, which produced motor activity, decreased femoral arterial resistance to 32 or 26% of pre-stimulation values, respectively. These data indicate that the posterior medial nucleus accumbens and/or substantia inominata may be involved in the control of peripheral cardiovascular changes associated with locomotion in dogs.

468.4

LOCALIZATION OF VAGOINHIBITORY CARDIOACCELERATORY AREAS IN THE BRAIN STEM OF CATS. S. J. Yeh* and J. S. Kuo* (SPON: J. T. Pan). Dept. of Internal Medicine and Medical Research , Taichung Veterans General Hospital, Taiwan, R.O.C..

In this study, electrical stimulation over the entire caudal brain stem was carried out to localize the cardioacceleratory sites in chloralose-urethane anesthetized and C2-3 spinal transected cats. Cardioacceleratory response was elicited in the ventromedulla (VM) at 2-4 mm rostral to the obex and 2.5-4 mm lateral to the midline, dorsalmedial medulla (DMM) including the prepositus hypoglossi and adjacent structures, and paramedian reticular nuclei (PRN) at 0-2 mm rostral to the obex and 1-2.5 mm lateral to the midline. The maximal response of stimulation was elicited at electric parameter of 2-4 V, 60-100 HZ and 1-2 ms. The tachycardiac response induced by stimulating one side of VM was slightly reduced and completely abolished by unilateral and bilateral vagotomy respectively , but was not abolished after iv infusion of Propranolol 1mg/kg or Tracrium 0.3 mg/kg. The results indicate that cells and/or fibers in VM, DMM and PRN may provide inhibitory inputs to both sides of the vagal nuclei and produce parasympathetic-inhibitory tachycardia.

468.5

BLOOD PRESSURE REGULATION, WEIGHT-CYCLING AND VASOPRESSIN IN RATS. P.F. Aravich & D.B. West. Depts. of Anatomy & Cell Biology, Physiology, & Internal Medicine, Eastern Virginia Med. Sch., Norfolk, VA 23501 and VA Medical Center, Hampton, VA 23667.

Repeated weight-cycling in rats has been reported to increase blood pressure compared to chronic overweight (Ernsberger & Nelson, AJP 254:R47). Further research is needed to confirm this effect. We tested the hypothesis that repeated fasting and re-feeding using a diet high in saturated fat and sucrose will increase mean arterial pressure (MAP) compared to chronic maintenance on this diet. The diet variable had two levels: A mixed-palatable diet (MPD) and a control (CON) diet. The MPD consisted of a defined, nutritionally complete solid component high in saturated fat (34.7% kcal lard) presented with a 20% sucrose solution; water was available ad lib. The CON diet was a modified AIN76A diet (11.6% kcal as corn oil) and had the same mineral/kcal ratio as the high-fat component. The cycling variable also had two levels: cycled (3, 3-day fasts separated by at least 9 days of re-feeding) and non-cycled. There were 4-6 male Sprague-Dawley rats in all conditions. It was found that neither retroperitoneal fat pad weight nor MAP (obtained from conscious, unrestrained rats) was differentially affected by the cycling condition. The MPD, on the other hand, increased both variables. Vasopressin levels as a function of diet and cycling will be reported later.

468.6

CHANGES IN CNS CATECHOLAMINE AND INDOLEAMINE METABOLISM ASSOCIATED WITH CHANGES IN BLOOD PRESSURE. J. Lovell, B.K. Yamamoto and S.L. Stuesse. Neurobiology Dept., N.E. Ohio Univ. College of Medicine, Rootstown, Ohio 44272.

Several interconnected CNS regions have been implicated in cardiovascular regulation. These areas include Nucleus Tractus Solitarius (NTS), Lateral Parabrachial Region (LPB), Bed Nucleus of Stria Terminalis (BNST), Lateral Hypothalamus (LH) and Central Nucleus of the Amygdala (CNA). We observed the changes in levels of the catecholamines Norepinephrine (NE) and Dopamine (DA) and the indoleamine Serotonin (5-HT) in these regions in response to changes in blood pressure. Urethane anesthetized male Sprague- Dawley rats (200-500gm) were placed under one of three experimental conditions: increased blood pressure (IBP), which received an i.v. bolus of phenylephrine; decreased blood pressure (DBP), which received an i.v. bolus of nitroprusside; controls, which received neither. Following i.v. injection, brains were rapidly removed and tissue samples from NTS, LPB, BNST, LH and CNA were analyzed for NE, DA and 5-HT content. Preliminary data indicate the following changes in NE, DA and 5-HT levels in response to changes in blood pressure: IBP rats have decreased DA and 5-HT in NTS, increased NE in BNST, while NE decreased in LH in IBP rats. DBP rats had decreased DA in CNA and decreased 5-HT in LPB, LH and CNA. These data display a site-specific differential role for NE, DA and 5-HT in response to different blood pressure conditions.

468.7

INCREASES IN ARTERIAL BLOOD PRESSURE AND DIFFERENTIAL BLOOD FLOW RESPONSES ELICITED FROM NEURONS IN THE DORSAL MEDULLA C.P. YARDLEY*, J.M. ANDRADE* and L.C. WEAVER (SPON:D.L. Jones). The John P. Robarts Research Institute and Dept. of Physiol., Univ. of Western Ontario, London, Ontario, Canada.

Neurons in the dorsal medulla (DM) formerly were considered important in the maintenance of arterial blood pressure (ABP). However, the DM recently has been suggested to be only a pathway from the rostral ventrolateral medulla (RVLM), a region crucial for this control (Brain Res.298:313, 1984). Experiments were done to search for sites in the DM at which chemical excitation of neurons could increase ABP and inhibition could decrease ABP. D,L,-homocysteic acid (DLH) or glycine was microinjected unilaterally into the DM of urethane or Saffan-anesthetized rats while recording ABP, heart-rate (HR), femoral and renal blood flow and conductance (RC, FC). At a number of sites dorsal to the RVLM, in the vicinity of the parvocellular reticular and dorsal paragigantocellularis nuclei, injection of DLH elicited increases in mean ABP (+5-60 mmHg) with variable heart-rate responses. At 7 sites, these pressor responses were accompanied by general vasoconstriction with decreases in both RC (-37%) and FC (-50%). However at 5 sites, RC decreased (-36%) but FC increased (+115%), indicating vasodilation in the hind-limb vascular bed. Injection of glycine occasionally decreased ABP but usually had no effect. These results show that neurons in this region of the DM can regulate ABP and control regional blood flow in a differential manner but are not usually crucial for support of basal ABP.(Support: Cdn. Heart Fdn.)

468.8

MODULATION OF REGIONAL BLOOD FLOW FOLLOWING ACTIVATION OF CENTRAL OPIOID RECEPTORS. A.H. Hassen, D. Brown* and E.P. Broudy*. Dept. of Physiology, W.V. School of Osteopathic Med., Lewisburg, WV 24901.

Prior studies demonstrated that microinjections of opioids into the dorsal vagal complex (DVC) elicited cardiovascular responses which were a function of receptor subtype and injection site. In the present study regional blood flow was measured with pulsed Doppler flowprobes or with radioactive microspheres following injection of small volumes (10 nl) of selective mu or kappa receptor ligands into the DVC. All experiments were performed on male Sprague-Dawley rats, anesthetized with pentobarbital, paralyzed with d-tubocurarine and artificially ventilated. Following mu receptor activation in the DMnV/nTS region BP and HR increased. Doppler measurements indicated decreased mesenteric artery flow, increased renal artery flow and no change in iliac flow. Vascular resistance was elevated in both the mesenteric and iliac arteries. Microsphere measurements revealed increased flow to the brain and kidneys, accompanied by decreased cardiac output and increased total peripheral resistance. These data demonstrate that activation of opioid receptors in the DVC can elicit a redistribution of cardiac output. These studies were funded by NIH grant NS 26147.

468.9

POSSIBLE BRAINSTEM SITES INVOLVED IN THE MEDIATION OF THE CENTRAL ANGIOTENSIN II (ANG-II) PRESSOR RESPONSE. L.R. Portis* and M.J. Brody (SPON: E. Anderson). Dept. of Pharmacol. and C.V. Center, Univ. of Iowa., Iowa City, IA 52242.

Our previous studies have shown that anesthetization of rostral ventrolateral medulla (RVLM) and rostral ventromedial medulla (RVMM) with the local anesthetic lidocaine (LIDO) attenuates the pressor response produced by intracerebroventricular (ICV) administration of ANG-II. The present study was designed to determine whether other brainstem sites (caudal ventrolateral medulla (CVLM), caudal ventromedial medulla (CVMM), and nucleus ambiguus (NA)), might also be involved in mediating this response. Experiments were performed on urethane anesthetized rats instrumented for measuring arterial pressure (AP) and heart rate (HR) and ventilated artificially. Bilateral injection of 200 nl LIDO in CVLM did not significantly change AP or HR. In contrast LIDO microinjected in CVMM produced a significant depressor response with no change in HR. The pressor response produced by ICV administration of 200 ng ANG-II was not affected by anesthetization of these sites or by LIDO injection in NA. These data suggest that unlike RVLM and RVMM, CVLM, CVMM, and NA appear not to be involved in the ANG-II central pressor response. The absense of effects in CVLM, CVMM, and NA emphasizes site-specificity of LIDO in the closely located regions of RVLM and RVMM.

468.10

CENTRAL NERVOUS SYSTEM EFFECTS OF CORTICOTROPIN-RELEASING FACTOR ON CARDIAC OUTPUT. J.M. Overton, G. Gorman*, and L.A. Fisher. Department of Pharmacology, College of Medicine, University of Arizona Health Sciences Center, Tucson, AZ 85724.

Central nervous system (CNS) administration of corticotropin-releasing factor (CRF) elevates systemic arterial pressure (AP) through alterations in autonomic nervous system activity. Furthermore, CRF produces concomitant mesenteric vasoconstriction and iliac vasodilation. However, the question of whether this pressor response results from increases in cardiac output versus total peripheral resistance, or both in combination has not been directly assessed. Male Sprague-Dawley rats (n=9) were instrumented with lateral intracerebroventricular (icv) cannulae, iliac arterial cannulae, and pulsed Doppler flow probes on the ascending aorta. Probes were attached to the aorta using cynoacrylic glue. Arterial pressure (AP), heart rate (HR), and ascending aorta flow velocity (Q) responses to icv administration of CRF (0.15 nmol) and saline (10 μl) were determined in conscious, unrestrained rats. Percent changes in Q, stroke volume (SV) and total peripheral resistance (TPR) were calculated. Icv administration of CRF elicited significant ($P<0.05$) and sustained (for 60 min) elevations of AP (12-20 mmHg), HR (50-100 bpm), and Q (25-30 %). Moderate reductions (5-10%) of TPR were observed after CRF treatment whereas SV was not significantly altered. Saline administration had no effect on the above cardiovascular parameters. These results indicate that central administration of CRF elevates AP primarily through increases in cardiac output. Taken together with previous findings, these data suggest a CNS action of CRF to produce enhanced AP and skeletal muscle perfusion supported primarily by augmentation of cardiac output.

468.11

CENTRAL NERVOUS SYSTEM CARDIOVASCULAR ACTIONS OF CORTICOTROPIN-RELEASING FACTOR: EFFECTS OF ANXIOLYTICS. L.A. Fisher, G. Gorman* and J.M. Overton. Department of Pharmacology, College of Medicine, University of Arizona Health Sciences Center, Tucson, AZ 85724.

Corticotropin-releasing factor (CRF) acts within the central nervous system (CNS) to increase sympathetic nervous activity, decrease parasympathetic nervous activity, elevate arterial pressure (AP) and increase heart rate (HR). Moreover, CRF acts within the CNS to produce behavioral activation indicative of enhanced fear. By virtue of its CNS distribution and actions, CRF is hypothesized to be a key physiologic mediator of the endocrine, autonomic and behavioral responses to stress. The anxiolytic agents, diazepam and alprazolam, are reported to suppress stress-induced activation of sympathetic nervous outflow. The present studies were aimed at determining whether these anxiolytic drugs also suppress CRF-induced sympathetic and cardiovascular activation. Conscious, unrestrained male Sprague-Dawley rats fitted with indwelling intracerebroventricular (icv) cannulae and iliac arterial catheters were used in all experiments. Rats received intraperitoneal injections of vehicle (1 ml/kg), diazepam (1 - 5 mg/kg) or alprazolam (0.5 - 3 mg/kg) thirty minutes prior to icv administration of saline (10 μl) or CRF (0.15 nmol). In rats receiving saline icv, diazepam and alprazolam treatments produced small elevations of baseline HR without affecting baseline AP. CRF-induced elevations of AP were attenuated after pretreatment with diazepam and alprazolam. In contrast, CRF-induced elevations of HR were not affected or were slightly enhanced by diazepam and alprazolam pretreatments. These data suggest that CNS-mediated cardiovascular activation by CRF is subject to modulation by benzodiazepine anxiolytics.

468.12

EFFECT OF NEUROPEPTIDES ON SYMPATHETIC PREMOTORNEURONS OF ROSTRAL VENTROLATERAL MEDULLA "IN VITRO". P.G.Guyenet and M.-K.Sun. Dpt. of Pharmacology,Univ. of Virginia,Charlottesville,Va 22908.

Spontaneously active cells (firing rate 3-12 spikes/s)of nuc. reticularis rostroventrolateralis (RVL) were recorded extracellularly in 500 μm thick rat brain slices maintained at 30°C (Sprague-Dawley, 70-120g).Previous experiments have suggested that i) these cells derive their resting activity from intrinsic pacemaker properties, ii) most are reticulospinal cells which provide an excitatory drive to vasomotor preganglionic cells and iii) they are not adrenergic.

These cells were uniformely excited by bath-applied argininvasopressin (AVP,57+10% at 1 μM).Using selective agonists and antagonists,the receptor subtype involved in the response to AVP was characterised as V1. The majority of these RVLneurons was also excited by NPY (25+9% at 0.01 μM,N=8),TRH (57+7% at 1μM),SubstanceP (30+10% at 1μM) and CGRP (36+11% at 0.04 μM). These RVL neurons were also inhibited by metenkephalin (30+6% at 1 μM) but they were unaffected by CRF (up to 0.1μM) or Angiotensin II (up to 1 μM).

In conclusion,the firing rate of the non-catecholaminergic subgroup of RVL sympathetic premotorneurons can be altered by several of the neuropeptides present in this nucleus.(HL 28785).

468.13

Effect of Naloxone Injections into Brainstem Nuclei on Baroreflex in Morphine-treated Rats. Karen L. Cochrane and Patrice G. Guyenet. University of Virginia, Dept. Pharmacology, Charlottesville, VA 22908.

Baroreflex slope (BRS, delta SNA per mmHg) was assessed before and after IV morphine (MOR: 8mg/kg)and after naloxone (NAL: .4μg) into the caudal (CVL) and rostral (RVL) ventrolateral medulla and IV (1mg/kg) in 6 rats anesthetized with halothane. The min and max levels of SNA obtained after increasing or decreasing MAP were defined as 0 and 100 units. MOR decreased BRS from 1.5±.2 to .3±.1 u/mmHg and resting (R) SNA from 84±-3 to 45±14u. The BRS and R-SNA were unaffected by NAL-CVL (.5±.1u/mmHg, 44±11u). NAL-RVL reversed BRS (1.8±.3 u/mmHg) and R-SNA (112±16u) and caused a rightward shift in the SNA vs MAP curve. NAL-IV did not alter BRS (1.8±.5u/mmHg), shifted the curve back to baseline and decreased R-SNA (59±13u). SNA range was dampened after MOR (7±2 to 52±9u) and NAL-CVL (16±4 to 77±10u) while NAL-RVL increased the max SNA (154±16u) beyond baseline. NAL-IV caused min SNA to decrease 29±8u below baseline. Endogenous opiates may act in the RVL to decrease BRS and basal level and range of SNA.

468.14

ELECTROPHYSIOLOGICAL CORRELATES OF THE CARDIOVASCULAR REGULATORY NEURONS OF THE ROSTRAL VENTROLATERAL MEDULLA. D. Bhaskaran, P. Patino* and C.R. Freed. Depts. of Med. and Pharm., Univ. of Colo. Health Sci. Ctr., Denver, CO 80262.

We have reported blood pressure (BP) mediated changes in concentrations of neurotransmitters in extracellular fluid of the rostral ventrolateral medulla (RVLM) (Neurosci. Abst. 227.12 (87); 82.14 (88). We have now studied changes in neuronal firing in RVLM in response to BP changes. Male Sprague-Dawley rats, 300-350 g, were anesthetized with urethane and the femoral artery and vein catheterized for BP measurement and drug administration. The floor of the IVth ventricle was exposed and glass electrodes filled with 2% pontamine blue were lowered into the RVLM. Once a neuron was found and baseline firing frequency measured, the BP was altered by either phenylephrine or nitroprusside infusions. Other rats were given clonidine (alpha 2-agonist) or yohimbine (alpha 2-antagonist). RVLM neurons located -3.72 to -3.8 mm from the interaural line increased firing during phenylephrine-induced hypertension. Cell firing fell during nitroprusside-induced hypotension. Neurons 1.0 mm rostral to this area did not respond to changes in BP. RVLM contains neurons which change firing in direct relation to induced changes in BP.

468.15

EFFECT OF SELECTIVE SURGICAL SA-NODAL PARASYMPATHECTOMY UPON THE HEART RATE RESPONSE TO A CONTROLLED BEHAVIORAL TEST. D.C. Randall, D.R. Brown*, W.C. Randall* and R.M. Raisch*. Dept. Physiol. & Biophys. & Ctr. Biomed. Engin., Univ. Kentucky, Lexington, 40536

The experiment was performed to quantify the role of the parasympathetic nerves to the SA node in controlling the heart rate (HR) response during Pavlovian conditioning. Dogs (n=5) were trained by following a 30 sec. tone (CS+) with a ½ sec. shock. Each animal's SA node was then selectively parasympathectomized (cf: Am. J. Physiol., 248:H61, 1985). Two weeks later they were retested in the behavioral paradigm before and after ß-blockade (propranolol, 1 mg/kg). The conditional HR response (CR) was analyzed in 5 sec. blocks. The table shows the average (± SEM) control (pre-CS+) HR (bpm) and peak conditional increase (CR: largest 5 sec. tachycardia during CS+) for pre-denervation (Pre), post-denervation (Post) and Post with ß-blockade (Post-ßB); "*" = $p < .05$ vs. "Pre":

Pre		Post		Post-ßB	
Control	**CR**	**Control**	**CR**	**Control**	**CR**
91 ± 10	42 ± 7	116* ± 5	44 ± 10	104 ± 6	11* ± 3

The maximal conditional increase in HR was not different pre- vs. post-denervation; conversely, the time from CS+ onset to peak HR was prolonged in 4 of 5 dogs. We conclude that the magnitude of the CR was determined primarily by increased cardiac sympathetic drive; parasympathetic factors significantly influence the time course of the conditional HR response. (Funded by HL 19343 & HL 27595)

468.16

AUTORADIOGRAPHIC ANALYSIS OF DIFFUSION OF SUBSTANCES MICROINJECTED INTO THE BRAIN. J.E. Kauth,* B. Knosp,* and W.T. Talman. (SPON: R. Lim). Lab. of Neurobiol. & Image Analysis Facility, Univ. of Iowa, Iowa City, IA 52242.

Microinjection of L-glutamate (GLU) into the nucleus tractus solitarius (NTS) of rat decreases arterial pressure (AP) and heart rate (HR). The site at which the agent acts has largely been determined by localization of the diffusion of a vital stain injected at the same site. We have, therefore, sought to assess directly the diffusion of GLU labelled with a tracer amount of [^{3}H]-GLU by a quantitative autoradiographic technique. Microinjections were made into the brain stem through glass micropipettes in anesthetized rats instrumented for recording AP and HR. After microinjection (10-50 nl), the animal was killed with i.v. KCl, the brain removed and frozen, and the brain stem cut in 25 um transverse sections that were desiccated and fixed with formaldehyde vapor. Autoradiograms were made and the slides were stained for microscopic examination. The histologic image and autoradiogram were digitized and oriented by fiducials. Nuclear groups were outlined, the orientation in 2 and 3 dimensions of the diffusion sphere to the nuclei determined, and the concentration of the agent in the diffusion rings around the center of the injection assessed. (Support: VA Merit Review, HL32205, HL14388, and NS24621.)

468.17

CARDIOVASCULAR EFFECTS OF MICROINJECTIONS OF ARGININE VASOPRESSIN INTO THE AREA POSTREMA. C.L. Beck*, M. Appalsamy*, R. Mosqueda-Garcia, D. Robertson. (Spon:R.A. Margolin) Dept. of Pharmacology, Vanderbilt University, Nashville, TN 37232.

Immunohistochemical evidence has demonstrated the presence of vasopressin (AVP) receptors in different parts of the lower brainstem. The area postrema (AP) is in a unique position to be stimulated by both central and peripheral influences. The cardiovascular effects of AVP microinjections in the AP have not been studied. In the present study, we investigated the hemodynamic effects of microinjected doses of AVP into the AP in normotensive rats.

Male Sprague-Dawley rats were anesthetized with urethane and changes in blood pressure (BP) and heart rate (HR) were recorded through a cannula placed in the femoral artery. After limited occiptal craniotomy, the AP was located by stereotaxic coordinates and by the pharmacological response to microinjected adenosine.

Different doses of AVP (0.125 ng to 20 ng) were microinjected into the AP in volumes not exceeding 60 nl. Low doses of AVP showed mainly depressor effects (-10±5 mmHg MBP/-21±5 b/m HR for 0.125 ng; n=3); intermediate doses showed biphasic (pressor-depressor) effects, and high doses showed mainly pressor effects (+12±4 mmHg MBP/+9±6 b/m HR for 20 ng; n=7). Microinjections of equivalent volumes of normal saline showed no response.

These results indicate that microinjection of AVP into the AP is depressor at lower dosages, but predominantly pressor at the highest dosages. These observations are consistent with the view that the AP is involved in the mediation of vasopressin effects on cardiovascular regulation.

468.18

INCREASED HYPOTHALAMIC AND MEDULLARY NOREPINEPHRINE RELEASE IN RESPONSE TO HEMORRHAGE IN THE CONSCIOUS RAT. J.W. Van Huysse* and S.L. Bealer, Dept. of Physiol., Univ. of Tenn., Memphis, TN 38163.

The responses of extracellular norepinephrine (ne) levels in the dorsomedial medulla (DMne) and paraventricular/anterior hypothalamic area (P/Ane) to maneuvers causing sympathetic activation were examined in conscious rats. DMne and P/Ane were estimated by in vivo microdialysis before and during hemorrhage (HEM, to a constant arterial pressure of 75 mm Hg), after reinfusion of HEM blood, or before and after i.v. hypertonic saline (HTS, 1.5 M NaCl at 10 ul/100g/min). Since lesions of the anteroventral third ventricle (AV3V) affect brain ne release (Am. J. Physiol. 256, R487), the responses of DMne and P/Ane to HEM or HTS were compared in rats with prior AV3V lesions (AV3V-X) or control surgery (CONT). DMne and P/Ane increased during HEM and returned to baseline after reinfusion of blood in both AV3V-X and CONT rats, but P/Ane responses to HEM in AV3V-X rats were greater than those in CONT rats. HEM volumes and DMne responses were similar in CONT and AV3V-X rats. HTS did not change DMne or P/Ane in AV3V-X or CONT rats, despite increases in plasma osmolality of ~35 mOSM. We conclude that 1) DMne and P/Ane increase in response to HEM, but not to HTS, and 2) the AV3V region appears to inhibit P/Ane, but not DMne responses to HEM (Supported by USPHS grant HL 25877 and NRSA HL07770).

468.19

CARDIAC EFFECTS OF NEUROPEPTIDE Y (NPY) OR CARBACHOL (CARB) FOLLOWING INJECTION INTO THE POSTERIOR HYPOTHALAMIC NUCLEUS (PHN). J.R. Martin, M.M. Knuepfer, and T.C. Westfall. Kirksville Coll. Osteo. Med., Kirksville, MO 63501 and St. Louis Univ. Sch. Med., St. Louis, MO 63104.

Injection of NPY or CARB into the PHN of conscious Sprague-Dawley rats results in an increased mean arterial pressure (MAP). The purpose of the present study was to determine the effect of administration of NPY or CARB into the PHN on various cardiac parameters. Rats were instrumented for arterial pressure and cardiac output measurement. A cannula was targeted toward the PHN for injection of NPY (10 mcg) or CARB (1 mcg). The increase in MAP evoked by CARB was paralleled by an increase in total peripheral resistance (TPR). Cardiac output (CO) was unchanged following CARB due to a decreased heart rate (HR) and an increased stroke volume (SV). The increase in MAP evoked by NPY was accompanied by an increase in TPR which persisted despite a return of blood pressure toward baseline levels. NPY decreased CO while increasing HR thereby causing a decreased SV which, like TPR, persisted. The changes in MAP and TPR, TPR and SV, and SV and HR evoked by CARB were highly correlated, while the changes in TPR and CO, TPR and SV, and CO and SV evoked by NPY were highly correlated. These results provide further evidence that NPY and CARB differentially affect the cardiovascular system following injection into the PHN. (Supported by HL26319, HL35202, NS07254, HL38299 and an AHA MO Affiliate Fellowship.)

468.20

INTERACTIONS OF THE TRACTUS SOLITARIUS AND THE AREA POSTREMA IN THE NUCLEUS OF THE SOLITARY TRACT. M. Hay* and V. S. Bishop. Department of Pharmacology, The University of Texas Health Science Center, San Antonio, TX 78284-7764

Previous *in vitro* studies from our laboratory have shown that electrical stimulation of the area postrema increased neuronal activity in the medial nucleus of the solitary tract (mNTS) and that this activation was possibly due to alpha-2 adrenoreceptor activation. The purpose of the present study was to investigate the interaction of afferent solitary tract stimulation with area postrema stimulation on the activity of mNTS neurons. Using an *in vitro* rabbit brain slice preparation, extracellular recordings were made from 30 mNTS cells of which 18 received both area postrema and solitary tract input. In these cells, simultaneous stimulation of the solitary tract and the area postrema at voltage levels which evoked no action potentials when stimulated separately resulted in the production of either single or multiple action potentials in mNTS neurons. The maximal temporal separation of the two stimuli which could still produce an action potential in the mNTS neurons was approximately 10 msec. To determine if the interaction between solitary tract afferents and area postrema afferents involved a possible facilitation we designed the following protocol. Stimulation levels of the solitary tract and the area postrema were adjusted so that the respective separate stimulations produced mNTS action potentials 30% of the time. Simultaneous stimulation of the area postrema and the solitary tract resulted in action potentials 94 ± .3% of the time. This response was 25% greater than the calculated summation of the separate responses. These studies suggest that solitary tract and area postrema afferents interact at the level of the mNTS and that this interaction appears to be facilitory. (Supported by HL12415 and HL36080).

468.21

CAPSAICIN SENSITIVE NEURONS IN RENAL PELVIS. U.C. Kopp and L.A. Smith*. Univ. Ia Col. Med., & VAMC, Iowa City, IA 52242

In rats stimulation of renal mechanoreceptors by increased ureteral pressure (↑UP) or chemoreceptors by renal pelvic perfusion with 0.9 M NaCl increases ipsilateral afferent renal nerve activity(ARNA), decreases contralateral efferent RNA and increases contralateral urinary sodium excretion; a contralateral renorenal reflex response. Chronic treatment with capsaicin, 950 mg/kg/1 week sc, abolished the renorenal reflex, suggesting that capsaicin sensitive neurons are involved. In the kidney, substance P (SP) immunoreactive neurons are located in the pelvic wall. Therefore, we compared the ARNA responses to capsaicin injected into renal pelvis and renal interstitiulm. Capsaicin injected into renal pelvis, 0.5, 5, 50, 500 and 5,000 ng (50 μl volume), increased ARNA dose dependently (integrated voltage, % of control): 60±19, 122±34, 245±69, 299±87 and 333±105% (N=6). Blood pressure increased 3 to 9 mmHg and heart rate fell 7 to 13 bpm. Capsaicin injected into renal interstitium, 50, 500, 5,000 and 50,000 ng, increased ARNA 52±21, 110±49, 168±66, 281±114%. Thus, for a given ARNA response, 100 times higher dose is required when capsaicin is injected into renal interstitium compared to renal pelvis. ↑UP 30 mm Hg increased ARNA 30±10%. SP into renal pelvis, 100 and 1,000 ng, increased ARNA 52 and 121%. These data suggest that the sensory neurons involved in renorenal reflexes are located in the renal pelvic wall and activated by capsaicin and SP.

468.22

SURGICAL STRESS ABOLISHES DIFFERENCES BETWEEN DIABETIC AND CONTROL RATS IN CENTRAL NEURAL PHENYETHANOLAMINE N-METHYL TRANSFERASE (PNMT) ACTIVITY. T.G. Campbell*, J.W. Manning, and J.K. Stewart, Dept. of Biology, Virginia Commonwealth Univ., Richmond, VA 23284 and Dept. of Anesthesia Research, Emory Univ., Atlanta, GA 30322.

Previously we showed that PNMT activity is elevated two fold in the medulla/pons of diabetic rats compared to that in controls. We now report that bilateral carotid ligation or sham surgery abolished this difference in enzymatic activity. Male Sprague Dawley rats were treated with streptozotocin or vehicle and maintained for 4 wks. All animals were anesthetized with sodium pentobarbital and allowed to recover from sham surgery or carotid ligation for 6 hr. PNMT activity ranged from 3.25 - 3.37 pmol/(hr x mg protein) in ligated and sham-operated controls and diabetics. These findings suggest that PNMT activity in diabetic animals may be maximal and cannot be further elevated by surgical stress. Furthermore, carotid ligation had no acute effects on medullary PNMT activity in control or diabetic animals.

468.23

CARDIORESPIRATORY RESPONSES TO ELECTRICAL STIMULATION OF THE DORSOMEDIAL HYPOTHALAMUS OF THE RABBIT. C.G. Markgraf, R.W. Winters*, P.M. McCabe, Y-F. Duan*, and N. Schneiderman. Dept. of Psychology, Univ. of Miami, Coral Gables, FL 33124.

The defense reaction is as an integrated pattern of cardiovascular and behavioral responses characterized by increases in heart rate (HR), blood pressure (BP), hindlimb blood flow (BF) and respiration rate (RESP). Stimulation of regions of the hypothalamus and midbrain in the cat and rat have been shown to produce these characteristic changes. Previously we have shown in the rabbit that injections of HRP into the rostral ventral lateral medulla (RVLM), a medullary area involved in control of BP, leads to retrograde labeling of cell bodies in the midbrain periaqeductal gray (PAG) and dorsomedial hypothalamus (DMH). We have demonstrated that electrical stimulation of the PAG elicits components of the defense reaction, possibly via these monosynaptic connections.

The present study assessed the cardiovascular and respiratory responses elicited by electrical stimulation of the DMH in the urethane anesthetized rabbit. Electrical stimulation (10 sec train, 100 Hz, 80-200 μA) of this region produced a response profile that was characteristic of the defense reaction: significant increases in BP (+11-15 mm Hg), HR (+15-22 bpm), BF (+3.0-4.5 cc/min) and RESP (+20-30 breaths/min) were observed. Taken together with results of previous studies, these data suggest that the DMH and the PAG are involved in the mediation of the defense reaction in the rabbit. The BP component of this response may be influenced by projections from these structures to the RVLM, which in turn projects to the intermediolateral cell column of the spinal cord. Supported by NIH grants HL 07426, HL 36588 and NS 24874.

468.24

RESPONSES OF CARDIAC RECEPTORS TO CHEMICAL AND MECHANICAL STIMULATION. R. Hainsworth*, F.M.S. Care*, D.S. Coulshed* and P.N. McWilliam* (SPON: Brain Research Association). Dept of Cardiovascular Studies, Univ. of Leeds, Leeds LS2 9JT, U.K.

This study was undertaken to determine whether afferent cardiac nerves which respond to chemical and mechanical stimulation of cardiac receptors are different.

In ten anaesthetized dogs, a cannula tied into the ascending aorta connected to a pressurized bottle, controlled left ventricular systolic pressure; diastolic pressure was held constant by a partial bypass. The cephalic circulation was perfused at constant pressure and a hind limb and the remainder of the circulation were perfused at constant flows so that changes in perfusion pressures denoted vascular resistance responses.

A step increase in aortic root and ventricular systolic pressures resulted in consistent vasodilatation, but no consistent change in heart rate. Injection into the aortic root of veratidine (10-25 μ g) and capsaicin (50-100 μ g) resulted in similar vascular responses but also consistently resulted in bradycardia. Following repeated injections or infusion of veratidine, the responses to further injections of veratidine or capsaicin were significantly reduced but those to changes in ventricular pressure were not significantly changed.

We conclude that, because the responses to chemical and mechanical stimulation of the ventricular receptors are different and because we could selectively desensitize the nerves mediating the chemical responses, it is likely that chemical and mechanical stimulation affect different populations of nerves.

468.25

EFFECT OF SUBSTANCE P ON RABBIT CAROTID SINUS BARORECEPTORS AND CHEMORECEPTORS. Q. LONG AND S.L. STUESSE. NEUROBIOLOGY DEPT., N.E. OHIO COLLEGE OF MED., ROOTSTOWN, OH 44272

Substance P (SP) is abundant in the carotid sinus nerve (CSN) and has been implicated in baro- and chemoreceptor reflexes. There has been disagreement about the effect of SP on the baroreflex and CSN single unit discharges. We examined the effect of SP on blood pressure and heart rate in urethane-anesthetized, spontaneously breathing rabbits with bilaterally cut cervical sympathetic nerves. SP (1ug/ml, 0.2ml total) or saline was slowly injected into the left internal carotid artery. SP decreased blood pressure from 104.5 $\pm$ 6.5 to 32.7 $\pm$ 5.1 mmHg ($P<0.001$, N=8) and heart rate from 326.8 $\pm$ 12.6 to 277.1 $\pm$ 20.8 beats/min. In 8 other rabbits, the carotid sinus area (CS) was vascularly isolated and perfused over 5 min. with SP in Locke's solution (1ug/ml, 0.2ml/min.) or Locke's alone. Arterial blood pressure, intrasinus pressure, heart rate, and unit activity from the CSN were recorded. SP decreased mean arterial blood pressure from 91.3 $\pm$ 12.3 to 74.5 $\pm$ 10.1 mmHg. This change is less than that obtained when we injected SP arterially. Heart rate was also significantly decreased from 320.2 $\pm$ 15.3 to 297.8 $\pm$ 15.5. SP increased the single unit activity of 12 of 18 baroreceptor fibers but inhibited all of 20 chemoreceptor fibers (decreased to 81% of control). The inhibition of chemoreceptive fibers may be direct or indirect due to dilation of smooth muscle. However, SP may directly activate CS baroreceptors and initiate a baroreflex.

468.26

INHIBITION OF THE BAROREFLEX BY GROUP III AND IV HIND LIMB AFFERENTS IN THE CAT. P.N. McWilliam* and T. Yang*. Dept. of Cardiovascular Studies, Leeds University, Leeds, U.K.

Stimulation of hind limb nerves in the cat will inhibit the cardiac vagal component of the baroreflex and limited evidence suggests that this effect is mediated by group IV or C fibres (Quest,J.A. & Gebber, G.L., *Am. J. Physiol.*, 222:1251, 1972). The present study re-examines the effects of other groups of hind limb afferents and was carried out on cats decerebrated under halothane anaesthesia. Carotid sinus baroreceptors were stimulated with saline injected through double lumen catheters inserted into the external carotid arteries (common carotid arteries snared) and pressure in both carotid sinuses was monitored. The reflex effect on the heart was expressed as the maximum prolongation of the R-R interval compared to the mean of ten R-R intervals before pressure elevation. Elevation of sinus pressure 0.7±0.2s after the onset of stimulation (5-10 Hz) of group I and II fibres in the peroneal nerve produced prolongations of pulse interval (171±113 ms) which were not significantly different from control. In contrast, when the stimulus intensity was increased to recruit group III fibres, conducting at 23.0 m/s, the prolongation of pulse interval was reduced to 52±15 ms. Recruitment of group IV fibres had a significantly greater effect, reducing the prolongation of pulse interval to only 1±9 ms. In all cases the carotid sinus baroreceptors were stimulated within one second of peroneal nerve stimulation and before any change in arterial pressure resulting from nerve stimulation. It is concluded that electrical stimulation of group III and IV fibres of the peroneal nerve attenuates the cardiac vagal component of the carotid sinus baroreflex.
(SPON: Brain Research Association).

468.27

CHANGES IN SYMPATHETIC NERVE ACTIVITY DURING ACUTE HEMORRHAGIC HYPOTENSION AND AFTER NALOXONE. J.C.Schadt and E.M.Hasser. Dept. Vet. Biomed. Sci. and Dalton Res. Center, Univ. Missouri, Columbia, Missouri 65211

The onset of hypotension during hemorrhage is accompanied by decreases in vascular resistance, plasma NE, and sympathetic nerve activity. Naloxone reverses these effects. Our hypothesis was that the increase in sympathetic nerve activity was specific to naloxone treatment and not due only to the increase in mean arterial pressure (MAP). Male, New Zealand white rabbits were chronically prepared with indwelling arterial and venous catheters and renal sympathetic nerve recording electrodes. The experimental protocol was to remove venous blood until MAP decreased to < 40 mmHg, inject naloxone (3 mg/kg) or saline, and monitor recovery for 5 min. Prehemorrhage MAP and heart rate (HR) were 73±3 mmHg and 181±12 beats/min, respectively. Nonhypotensive hemorrhage did not change MAP but increased HR to 250±21 beats/min and renal sympathetic nerve activity (RSNA) to 248±39% of control (N=6). During hypotensive hemorrhage, MAP and HR decreased to 30±1 mmHg and 228±21 beats/min, respectively. RSNA decreased to 10±8% of control. Naloxone injection (N=3) increased MAP (after 2 min) to 80±1 mmHg and increased RSNA to 215±34% of the prehemorrhage control. Two min after saline injection, MAP was 42±4 mmHg and RSNA was 41±18% of control. α-adrenergic blockade with prazosin (N=3) reduced the pressor response to naloxone so MAP was only 48±1 mmHg 2 min after injection. RSNA increased to 426±47% of prehemorrhage control. Thus, pretreatment with prazosin reduced the pressor response to naloxone but appeared to potentiate the increase in RSNA. We conclude that opioid receptor blockade with naloxone during acute hemorrhagic hypotension increases sympathetic nerve activity. This increase in sympathetic nerve activity is not dependent on the increase in MAP. Rather, the increase in sympathetic nerve activity accounts in large part for the increase in MAP. Supported in part by BNS-8719372, HL31218, and HL36080.

468.28

CARDIOMOTOR INHIBITION IN CONGESTIVE HEART FAILURE: INITIAL LOW HEART RATE VARIABILITY IN SUBJECTS AT RISK FOR SUDDEN DEATH ASSOCIATED WITH BRADYARRHYTHMIA. R.B. Trelease*, R.M. Harper, W. Stevenson*, L. Stevenson*, M. Woo* and D. Heaney*. (SPON: P. Kaufmann). Department of Anatomy and Cell Biology and Cardiology Division, UCLA School of Medicine, Los Angeles, CA 90024.

Cardiac arrhythmia can be evoked by stimulation of reflexes and central nervous system activity, as well as by pathophysiologic mechanisms within the myocardium. Ventricular fibrillation, an arrhythmia commonly associated with sudden death, has been linked to sympathetic cardiomotor activity and particularly to lateralized (left stellate ganglion) hyperactivity. However, a recent study of subjects with severe congestive heart failure (CHF) has demonstrated a high rate of sudden death associated with bradyarrhythmia, suggesting a vagally mediated inhibitory process. To assess the relative roles of parasympathetic and sympathetic innervation in such lethal arrhythmia, we have begun prospectively recording high-resolution EKGs, respiration, and sleep-state variables (EEG, EOG, etc.) in subjects with severe CHF in the UCLA Heart Failure program. Initial analysis of heart rate variability data confirms reduced sinus arrhythmia (SA) previously reported in CHF. Two basic patterns have emerged: 1) low respiratory (vagal) SA alone, and 2) low overall SA with the virtual absence of low-frequency (sympathetic) components as well. The initial low respiratory SA suggests, in the absence of functional denervation, the strong inhibition of vagal cardiomotor activity in severe CHF. It is possible that potentially lethal bradyarrhythmia may be allowed by the return of previously inhibited vagal activity during hemodynamic fluctuations with treatment for CHF.

469.1

PHARMACOLOGIC AGENTS WHICH ANTAGONIZE ACUTE AMPHETAMINE TOXICITY R. Derlet*, T. Albertson. Div. Emergency Medicine/Clinical Toxicology, UC Davis, Medical Center, Sacramento, CA 95817.

Several agents were tested to determine their efficacy in preventing seizures and death induced by d-amphetamine in rats. A lethal dose of amphetamine (75mg/kg) was administered intraperitoneally to rats. Ninety-two percent of control animals developed seizures in a mean time of 13.3 ± 0.5 min., and 100% of animals died in a mean time of 55.0 ± 9.0 min. Animals were pretreated with test agents 30 minutes prior to administration of the amphetamine in order to insure adequate systemic absorption. In this model, haloperidol in a dose range 1.0-20mg/kg showed statistically significant efficacy in reducing death ($P<0.05$). Propranolol afforded significant efficacy at doses of 20 and 30mg/kg ($P<0.05$). A combination of haloperidol (1.0mg/kg) and propranolol (10mg/kg) completely prevented death. Animals pretreated with diazepam (1.0 to 10mg/kg), yohimbine (2.5 and 10mg/kg), or prazocin (5 and 10mg/kg) had no significant difference in death incidence compared to controls. Diazepam (5 and 10mg/kg) was the only agent to reduce the incidence of seizures.

469.2

PCA- OR MDMA-ENHANCED STARTLE REFLEXES IN RATS: EFFECTS OF THE 5-HT UPTAKE BLOCKER MDL 27,777, SELECTIVE RAPHE LESIONS, OR PRIOR 5-HT DEPLETION. JH Kehne, TC McCloskey, VL Taylor, C Black, and CJ Schmidt. Merrell Dow Research Institute, 2110 E. Galbraith Road, Cincinnati, OH 45215.

The relationship between the immediate releasing effects of substituted amphetamines (e.g. PCA or MDMA) and the long-term neurotoxicity on 5-HT terminals is not clear. Serotonin uptake inhibitors antagonize MDMA-induced 5-HT release and reverse both the acute neurochemical and chronic neurotoxic effects of MDMA. Acute behavioral effects of PCA and MDMA include enhanced acoustic and tactile startle reflexes in rats. We now show that MDL 27,777 (5 mg/kg, ip), a 5-HT uptake inhibitor (J. Freedman et al., abstract this meeting) prevents the startle-enhancing effect of MDMA (20 mg/kg, sc). Furthermore, 4 weeks after initial injections of PCA or MDMA, startle amplitude still increased in response to a second challenge injection of 10 mg/kg PCA. This second dose did not further deplete 5-HT. Finally, selective radiofrequency lesions of either the dorsal or median raphe nuclei did not block the startle enhancing effect of PCA nor did they prevent further loss of 5-HT by PCA. These results indicate that the acute startle-enhancing effects of PCA and MDMA are attributable to 5-HT release from neurons that are (1) not of dorsal or median-raphe origin, and (2) insensitive to PCA toxicity. Studies are in progress to investigate the anatomical sites and mechanisms underlying the startle-enhancing effects of MDMA.

469.3

REVERSAL OF THE ACUTE EFFECT OF METHYLENEDIOXYMETHAMPHETAMINE (MDMA) BY 5-HT UPTAKE INHIBITORS. C.J. Schmidt,* C.K. Black* and V.L. Taylor* (SPON: P. Robinson). Merrell Dow Research Institute, 2110 E. Galbraith Road, Cincinnati, OH 45215.

Administration of MDMA to rats produces an acute decline in central 5-HT concentrations. This depletion is due to the rapid, carrier-mediated release of 5-HT coupled with a V_{max} loss of tryptophan hydroxylase (TPH) activity. Recent evidence suggests that the latter may be due to the oxidation of critical -SH groups in the enzyme. This being the case, we reasoned that interference with the ongoing process leading to oxidation may allow endogenous antioxidants to regenerate active TPH. Rats were administered MDMA (10 mg/kg, s.c.) followed by either saline or the 5-HT uptake inhibitor, MDL 27,777, 1 or 2 h later. Animals were killed at 1, 3, or 6 h for the determination of regional 5-HT concentrations and TPH activity. At 1 h, cortical 5-HT and TPH activity had declined to 45 and 58% of control, respectively. By 6 h these same values were 23 and 31%. In contrast, 5-HT concentrations were 85% of control in MDMA animals given MDL 27,777 at 1 h while TPH activity had returned to 92%. Similar results were observed in the hippocampus and striatum. These results suggest that mechanisms exist within the 5-HT neuron for the regeneration of inactivated TPH. If this inactivation is via oxidation, it is possible that the administration of exogenous antioxidants may reduce or block the loss of TPH activity produced by MDMA and related agents.

469.4

RAPID FORMATION AND DISAPPEARANCE OF THE MDMA METABOLITE, 3,4-DIHYDROXYMETHAMPHETAMINE BY RAT LIVER MICROSOMES. M. HIRAMATSU* AND A.K. CHO. Department of Pharmacology, UCLA School of Medicine, Los Angeles, CA 90024-1735.

The *in vitro* metabolism of (+) and (-)3,4-methylenedioxymethamphetamine (MDMA) by rat liver microsomes was examined. MDMA is metabolized to 3,4-methylenedioxyamphetamine and 3,4-dihydroxymethamphetamine by demethylation and demethylenation, respectively. A major metabolite after 2-3 min incubation was 3,4-dihydroxymethamphetamine. This reaction appears to be P450 dependent since it was very sensitive to SKF 525-A with Km and Vmax values of 2.7 μM and 617.1 pmoles for the (+) isomer and 1.5 μM and 428.3 pmoles for the (-) isomer. Methimazole did not inhibit this reaction. After 2-3 min, 3,4-dihydroxymethamphetamine was further metabolized to an unknown compound. This pathway requires oxygen and NADPH but seems not to be P450 dependent. (+) MDMA was more extensively metabolized to 3,4-dihydroxymethamphetamine than the (-) isomer. Since MDMA itself is not a toxic compound and studies of MDMA enantiomers have shown that the (+) isomer was more potent than the (-) isomer as a neurotoxin, this catechol or metabolite of this catechol may be involved in the toxicity of MDMA.

(Supported by USPHS grant DA 04206)

469.5

THE EFFECTS OF HABENULA LESIONS ON AMPHETAMINE-INDUCED BEHAVIOR AND GLUCOSE UTILIZATION IN RATS. B.Nguyen*, L. Jackson*, S. Caldecott-Hazard (SPON:J. Trubatch). LBES, UCLA, and Dept. Psychiatry, VAMC, Sepulveda, CA 91343.

Previous studies have shown that the administration of, or withdrawal from, amphetamine (AMP) alters glucose utilization in the habenula (HAB). Such data suggests a functional role for this brain area in AMP-induced behaviors. We tested this hypothesis by making bilateral lesions of the habenula. Rats with HAB lesions, and those with sham lesions, were either tested in an open field or were given 14C-2-deoxyglucose (2DG). Other groups of lesioned or sham rats were implanted (s.c.) with osmotic mini pumps containing AMP, 15mg/kg/day. After 7 days the pumps were removed, and 24 hours later rats were tested in the open field or given 2DG. The number of squares crossed in the open field was increased in rats with HAB lesions as compared to sham lesioned rats. HAB lesions also elicited changes in glucose utilization in several brain areas. During withdrawal from amphetamine (AMPWD), both lesioned and sham rats showed decreases in square crossing as compared to rats without AMP. Similarities in glucose utilization also occurred between lesioned and sham rats during AMPWD. The greatest differences between lesioned and sham rats were found during open field testing while AMP pumps were still implanted. Lesioned rats had increased square crossing and stereotypy as compared to sham rats. Thus, the habenula is involved with AMP-induced behaviors, but its role is a complex one.

469.6

SHORT-TERM VS. LONG-TERM BEHAVIORAL EFFECTS FOLLOWING CHRONIC ADMINISTRATION OF MDMA IN RATS. L.L. Wing, D.M. Downing and M.A. Geyer, Dept. Psychiatry, T-004, Univ. of California San Diego, La Jolla, CA 92093.

Sixty-two male Sprague-Dawley rats, 270-300g, were given a total of 8 subcutaneous injections, each given at 12 hour intervals over 4 consecutive days. Half the rats received MDMA (methylenedioxymethamphetamine;10mg/kg). This regimen is known to produce chronic depletions in serotonergic markers (Slikker et al, 1988; Stone et al, 1987). Half the rats received saline. Forty animals were tested 14 days after the first injection (the "long-term" group), while the rest were first tested at 6 days following their first-injection (the "short-term" group). The injection schedule for the long-term group began first so that all animals could be tested at the same time. All animals were tested for 1 hr in the Behavioral Pattern Monitor, a 1 X 2 ft box equipped with photocell beams arranged in an X-Y coordinate system around 3 inch centers.

Overall activity during the hour-long session was decreased in the short-term MDMA rats ($F(1,20)=15.90, p .001$). Crossovers between 6 inch squares was also significantly decreased ($F(1,20)=5.90, p .05$). Entries into the corner regions of the BPM were reduced ($F(1,20)=4.31, p=.05$), and a trend toward decreased entries into the center was observed ($F(1,20)=3.57, p=.07$). For long-term MDMA rats, no significant effects on these behavioral measures were observed.

Thus, short-term 5HT involvement in behavioral effects, with compensatory adaptation long-term, is suggested.

469.7

DIFFERENTIAL REACTIVITY TO ENVIRONMENTAL AND PHARMACOLOGICAL CHALLENGES PREDICTS INDIVIDUAL VULNERABILITY TO AMPHETAMINE SELF-ADMINISTRATION. P.V. Piazza*, J.M. Deminière*, M. Le Moal, H. Simon* (SPON: C.M. Thinus-Blanc).

The objective of the present work was to answer the following question: are there inherited or acquired predisposing factors indicative of the future susceptibility to amphetamine self-administration (SA) in rats? In view of the strong relationships between stress and the activity of dopaminergic neurons on one hand and between dopaminergic neurons and SA behavior, on the other, we studied two factors: the behavioral reactivity to stress and the alteration of dopaminergic neuronal activity by repeated stimulation with amphetamine injections (sensitization procedure).

We showed that Sprague-Dawley rats originating from the same breeding center exhibited a great variability in their locomotor response in a novel environment (scores from 200 to 1200 in 2 hours). This individual difference in the reactivity to a mild stress was positively correlated ($r=0.47$, $p<0.01$) to the individual sensitivity of DA neurons measured by the locomotor response to an i.p. injection (1.5mg/kg) of amphetamine. Moreover, the development of SA with low doses of amphetamine 10µg/injection) was more rapid in animals showing the more intense reactivity to the novelty stress ($r=0.78$; $p<0.001$). Finally, the repeated activation of DA neurons provokes the development of SA behavior in those animals previously hyporeactive to stress and to amphetamine.

These results may provide a psychobiological basis for addiction liability observed in human.

469.8

LOW-DOSE ETHANOL TREATMENT POTENTIATES D-AMPHETAMINE-PRODUCED CONDITIONED PLACE PREFERENCE. P.M. Duncan and C. Pelphrey-Weigand*. Psychology Department, Old Dominion University, Norfolk, VA 23508.

Interaction between ethanol (ETH) and d-amphetamine sulfate (DA) in the production of conditioned place preference (CPP) was studied by combining very low doses of ETH and DA in 4 groups of male rats (N=10 each). Treaments (IP injections, doses mg/kg) for each group: CTRL, saline; DA, DA .6; DAE6, DA.6+ETH60; DAE12, DA.6+ETH120. No ETH-only groups were included since these extremely low ETH doses produce no CPP effects. Rats were placed in one side of a shuttlebox (black-white striped walls) for 30 min immediately after drug treatment, and on alternate days placed in the other side (unpainted wooden walls) after saline injection. After 3 drug, and 3 nondrug conditioning days, preference for the two distinctive sides was tested (900-sec session). Mean total seconds spent in the non-drugged side per group: CTRL=440; DA=432; DAE6=294; DAE12=302. ANOVA revealed that DA-treatment (only) produced no CPP, but DA combined with either ETH dose did produce CPP (the combination groups' side preference was significantly different from CTRL and DA groups). Similar potentiation at these doses has been demonstrated for DA stimulus properties and may result from the dopamine-agonist effect of ETH.

469.9

INDIVIDUAL DIFFERENCES IN ACQUISITION OF AMPHETAMINE SELF-ADMINISTRATION IS CORRELATED WITH BEHAVIORAL AND ENDOCRINOLOGICAL RESPONSE TO STRESS. J.M. Deminière*, P.V. Piazza*, S. Maccari*, M. Le Moal, P. Mormède* and H. Simon* (SPON: J. Koenig). INSERM U.259, Domaine de Carreire, 33077 Bordeaux Cedex FRANCE.

Vulnerability to drug-intake shows individual differences between the rats of the same strain. In this work, we were interested in characterizing these differences both at the behavioral and biological level. The activity of the hypothalamo-hypophyso-corticoadrenal axis, as measured by the plasmatic concentrations of corticosterone, was studied in correlation with the sensitivity to stress and with the susceptibility to develop an amphetamine self-administration (SA) behavior. The results obtained were the following: 1) the rats with a low basal level of corticosterone were low-responders in a novel environment and did not acquire the SA behavior. On the contrary, rats with a high basal level of corticosterone showed a high locomotor response to novelty and rapidly acquired SA, 2) while the level of corticosterone returned to basal level 2 hours following exposure to novelty in low-responder rats, it attained more than 300% of the initial concentration in high responders. 3) Exposure to novelty immediately before the SA test increased the drug intake in rats. The characterization of typologies may be useful in identifying the individual at risk for the development of drug abuse.

469.10

THE EFFECTS OF NICOTINE, MIDAZOLAM, MORPHINE AND ETHANOL ON AMPHETAMINE STIMULUS GENERALIZATION FUNCTIONS J.P. Druhan, H.C. Fibiger and A.G. Phillips. Dept. Psychol.; Div. Neurol. Sci., Univ. of British Columbia, Vancouver, B.C., Canada, V6T 1W5.

Rats were trained to discriminate 1.0 mg/kg amphetamine (ip) from saline, and then tested for generalization to a range of amphetamine doses (0.0, 0.25, 0.50 & 1.0 mg/kg) injected either alone, or after pretreatments with nicotine, midazolam, morphine or ethanol. Nicotine (0.2 & 0.4 mg/kg, sc) both generalized partially and augmented the stimulus properties of amphetamine, so that the amphetamine stimulus generalization function was elevated relative to the curve obtained without nicotine. Midazolam (0.10 & 0.20 mg/kg, sc) and morphine (2.0 mg/kg, sc) attenuated the stimulus properties of amphetamine causing the generalization functions to be lowered relative to control curves. Ethanol (0.5 & 1.0 g/kg, ip) enhanced the cueing effects of amphetamine, but did not produce generalization when injected alone. These results demonstrate that the stimulus properties of amphetamine may be affected by psychoactive drugs from a variety of pharmacological classes.

469.11

SEQUELAE OF 3,4-METHYLENEDIOXYMETHAMPHETAMINE (MDMA) IN HEAVY USERS: PRELIMINARY FINDINGS. L.H. Price, M.D.,* G.A. Ricaurte, M.D., Ph.D., J.H. Krystal, M.D., G.R. Heninger, M.D. Dept. of Psychiatry, Yale Univ. Sch. of Med., 34 Park St., New Haven, CT 06508.

3,4-methylenedioxymethamphetamine (MDMA; "Ecstasy"), a selective serotonin (5-HT) neurotoxin in laboratory animals, has achieved notoriety as a recreational drug, and sone clinicians claim it has psychotherapeutic potential. We studied its effects on 5-HT function by comparing responses to the 5-HT precursor L-tryptophan (L-TRP) in MDMA users and healthy controls. Psychological effects of MDMA in users were also examined. Methods: 9 heavy MDMA users (mean ± S.D. total cumulative dose = 13.3 ± 13.4 g) participated. Last use was 66 ± 50 days before testing. After an overnight fast, subjects received L-TRP 7 g i.v. infused over 20 minutes. Serum prolactin (PRL) and subjective mood ratings were obtained before and after infusion. Age- and sex-matched controls were used for comparison. Results: In the controls, L-TRP caused significant increases over baseline in the peak PRL response (11.0 ± 13.1 ng/ml., $p<.008$) and the area under the curve (AUC) of the PRL response (568.8 ± 762.5 ng-min/ml, $p<.02$). In the MDMA group, peak change in PRL (5.9 ± 8.5 ng/ml, $p<.07$) and the AUC PRL response (224.8 ± 491.9 ng-min/ml, $p<.09$) failed to reach statistical significance. However, PRL responses between the control and MDMA groups did not reach significance, nor did mood responses differ between groups. MDMA was reported by users to have a unique profile of psychological effects reminiscent of both hallucinogenic and stimulant drugs. Conclusion: This study suggests 5-HT function may be diminished in MDMA users, consistent with findings in animals given comparable doses. Peak change in PRL response was 46% lower and AUC PRL response was 60% lower in MDMA users than in controls, but these differences between groups did not reach statistical significance. More definitive studies using larger samples are still needed.

469.12

A COMPARISON OF THE BEHAVIORAL AND NEUROCHEMICAL EFFECTS OF 5,7-DHT, MDMA AND d,l-FENFLURAMINE. N. Hata*, T. Cabrera*, and S. Lorens (SPON: R. Schmidt). Department of Pharmacology (Bldg. 135), Loyola University Medical Center, Maywood, IL 60153

Two experiments are in progress. In the first, separate groups (n = 8-12) of male Spraque-Dawley rats were treated with different doses of 5,7-dihydroxytryptamine (5,7-DHT; 0, 50 or 200 ug, i.c.v.; 30-45 min following nomifensine and desipramine, 15 mg/kg, i.p.), 3,4-methylenedioxymeth-amphetamine (MDMA; 0, 10 or 40 mg/kg, s.c., b.i.d. x 4 d), or d,l-fenfluramine (FEN; 0, 5 or 20 mg/kg, s.c., b.i.d x 4 d). Exploration of a novel open field (12 min), morphine analgesia (5.0 mg/kg, s.c.), and swimming ability (14 min) were examined on the three days immediately preceding sacrifice either two or eight weeks post-treatment. In the second experiment, separate groups of rats were treated with either saline (1.0 ml/kg, s.c., b.i.d. x 4 d), MDMA (40 mg/kg, s.c., b.i.d. x 4 d) or FEN (12 mg/kg, s.c., b.i.d. x 4 d). The animals were food deprived, maintained at 80-85% their pre-deprivation body weights, and trained in a 8-arm radial maze for food reinforcement.

The neurochemical analyses completed to date show that all treatments reduce hippocampal, neostriatal, nucleus accumbens and hypothalamic 5-HT levels two weeks post-administration, but that 5,7-DHT is the most effective. The 5,7-DHT treated rats tended to exhibit impaired swimming ability, and the highest dose (200 ug) may potentiate morphine analgesia eight weeks post-injection. Otherwise the 5,7-DHT rats appeared normal. MDMA and FEN treatment failed to affect any of the behaviors studied. These and previous data suggest that repeated high doses of MDMA and FEN do not lead to dysfunctions in exploratory behavior, motor coordination or stamina, the acquisition of a one- or two-way conditioned avoidance response, or spatial memory. (Supported by NIDA Contract #271-87-8117)

469.13

FLUNARIZINE BLOCKS THE DECREASE IN TRYPTOPHAN HYDROXYLASE ACTIVITY INDUCED BY 3,4-METHYLENEDIOXYMETHAMPHETAMINE. M. Johnson, K. Mitros*, G.R. Hanson and J.W. Gibb (SPON: J.R. Baringer), Dept. Pharmacol. and Toxicol., University of Utah, Salt Lake City, UT 84112.

N-methyl-D-aspartate (NMDA) receptors have recently been linked to the decrease in tryptophan hydroxylase (TPH) activity induced by multiple administrations of methamphetamine (METH) (Johnson et al.,1989). Because NMDA receptors are linked to a calcium channel, in this study we examined the ability of the calcium channel antagonist, flunarizine (FLU), to alter the changes in TPH activity induced by METH or 3,4-methylenedioxymethamphetamine (MDMA). Male Sprague-Dawley rats (180-240 g) received 4 doses of either METH (15 mg/kg, s.c.) MDMA (10 mg/kg, s.c.) or vehicle (0.9% NaCl) at 6-hr intervals. The animals treated with FLU (30 mg/kg, i.p.) received the drug 15 min prior to administration of NaCl , METH or MDMA. The animals were killed 18 hr after the last drug administration. METH and MDMA administration reduced neostriatal TPH activity to 64% and 38% of control, respectively. Coadministration of FLU with METH further reduced TPH activity to 40% of control while cotreatment of FLU with MDMA provided significant protection from the MDMA-induced decrease in TPH activity as enzyme activity was lowered to only 78% of control. These results suggest that calcium may participate in the MDMA-induced decline in central TPH activity, but the mechanism by which METH decreases TPH activity may differ from MDMA. (Supported by grants DA 00869, DA 04222 and MH 44454)

469.14

MK-801 ATTENUATES THE RESPONSE OF TRYPTOPHAN HYDROXYLASE TO METHAMPHETAMINE. K. Mitros*, M. Johnson, G.R. Hanson and J.W. Gibb, Dept. Pharmacol. and Toxicol., University of Utah, Salt Lake City, UT 84112.

Multiple administrations of methamphetamine (METH) induce a decrease in brain tryptophan hydroxylase (TPH) activity which is attenuated by administration of MK-801, a non-competitive antagonist of N-methyl-D-aspartate (NMDA) receptors. Interestingly, the changes induced by the METH analogue, 3,4-methylenedioxymethamphetamine (MDMA), were resistant to the MK-801 action. The purpose of this study was to further characterize these responses. Male Sprague-Dawley rats (180-240 g) received 4 doses of either METH (15 mg/kg, s.c.) MDMA (10 mg/kg, s.c.) or vehicle (0.9% NaCl) at 6-hr intervals. The animals treated with MK-801 (0.4 or 2.5 mg/kg, i.p.) received the drug 15 min prior to administration of NaCl , METH or MDMA. The animals were killed 18 hr after the last drug administration. METH and MDMA administration reduced neostriatal TPH activity by 50 to 80% of control. Coadministration of 0.4 mg/kg of MK-801 failed to alter METH-induced changes. While a 2.5 mg/kg dose of MK-801 attenuated the METH effect, both doses of MK-801 failed to alter the decline in TPH induced by MDMA. Administration of phencyclidine (20 mg/kg) mimicked the effect of MK-801 on the METH-induced decline in serotonin (5-HT) without altering the effect of MDMA on 5-HT levels. These results suggest that NMDA receptors may participate in the METH-induced change in the central 5-HT system, while their role in the MDMA-induced changes remains uncertain. (Supported by grants DA 00869, DA 04222 and MH 44454)

469.15

STEREOSPECIFIC EFFECTS OF MDMA (ECSTASY) AND MBDB ON CATECHOLAMINE RELEASE FROM PC-12 CELLS. S.T. Christian, F. Benington*, R.D. Morin*, J.M. Beaton, and J.A. Monti. Neuropsychiatry Research Program and Dept. of Psychiatry, U. of Alabama at Birmingham, University Station, Birmingham, AL 35294, U.S.A.

The effects of S-(+) and R-(-) MDMA and MBDB on catecholamine release from PC-12 cells was determined using drug concentrations of 1.0 to 500 μM, with quantification of released norepinephrine (NE) and dopamine (DA) by HPLC-EC. Dose-response curves of catecholamine release (% total cellular catecholamine content) versus drug concentration were constructed and EC-50's for MDMA and MBDB-stimulated release of NE and DA were determined:

Compound	EC-50 (μM)	
	NE	DA
S-(+)-MDMA	35	100
R-(-)-MDMA	150	100
S-(+)-MBDB	100	130
R-(-)-MBDB	400	130

The data indicate: (1) S-(+) and R-(-) MDMA are more potent in stimulating NE release than are S-(+) and R-(-) MBDB, respectively, and (2) S-(+) MDMA and MBDB are more potent in stimulating the release of NE as compared with DA, whereas the converse is true for R-(-) MDMA and MBDB. Further, at the lowest tested concentration of each drug, the amount of catecholamine released from the cells remained significantly higher than the amount of catecholamine released from the cells in the absence of drugs. It appears that: (1) There are differences in potency between S-(+) and R-(-) MDMA and MBDB in stimulating catecholamine release from PC-12 cells and (2) the mechanism(s) for MDMA and MBDB-stimulated catecholamine release from PC-12 cells may involve drug interaction(s) at more than one site. Supported in part by the Alabama Consumer Fund.

469.16

4-METHYLAMINOREX ("U4Euh"): A NEW DRUG OF ABUSE THAT PRODUCES AMPHETAMINE-LIKE STIMULUS EFFECTS. B Misenheimer* and R A Glennon*, Dept of Medicinal Chemistry; Virginia Commonwealth University, Richmond, VA 23298. (SPON: L. S. Harris)

2-Amino-4-methyl-5-phenyl-2-oxazoline or 4-methylaminorex ("U4Euh","ICE", 4-MAX) was originally developed as a potential anorexic in the 1960s. This agent is now appearing on the clandestine market and has been associated with a recent fatality. As of April 1989, 4-MAX is classified as a permanent Schedule I substance. Surprisingly, relatively little is known about the central effects of this agent

4-Methylaminorex possesses two chiral centers and exists as cis and trans geometric isomers. In this study, we evaluated, in tests of stimulus generalization, the effects of (±)cis, (+)cis, (-)cis, (±)trans, (+)trans, and (-)trans 4-MAX (15-min psii) in male S-D rats trained to discriminate 1.0 mg/kg of (+)amphetamine sulfate (i.p.) from saline in a standard 2-lever operant procedure under a VI 15-sec schedule of reinforcement for food reward. The cis racemate and its (+)- and (-)-isomers all produced (+)amphetamine-like responding; ED50 values are (±)cis: 1.5, (+)cis: 1.5, and (-)cis: 1.2 mg/kg, relative to (±)amphetamine: 0.7 mg/kg. The (+)amphetamine-stimulus also generalized to the trans isomer. The results of this study demonstrate that 4-methylaminorex can produce amphetamine-like stimulus effects in animals and that stereochemistry plays only a minor role.

469.17

DRUGS OF ABUSE PRODUCE PERSEVERATIVE BEHAVIOR IN THE RADIAL ARM MAZE. E.A. Loh, M. Sardelis* and D.C.S. Roberts Department of Psychology, Carleton University, Ottawa, Canada K1S 5B6.

The hypothesis that all drugs of abuse share the property of promoting perseverative behavior was investigated. Food deprived Wistar rats were permitted to explore an eight arm radial maze in which each arm was baited with one 45 mg food pellet. Unlike most other studies which have used this type of maze, the arms were re-baited each time the animal collected the food and returned to the center area. Therefore, every arm entry was reinforced and no "errors" were possible. Analysis of the sequences of arm entries demonstrated that drug naive rats showed no significant arm preferences or turning biases. However, when repeatedly pretreated with various doses of amphetamine, heroin or nicotine (but not saline) rats displayed repetitive patterns of arm entries which corresponded to a significant perseveration in angle of turn (rather than arm preference). Similar results have been reported with either ethanol or benzodiazepine injections. The data demonstrate that reinforcing drugs, from a variety of pharmacological classes, produce an increase in perseverative behavior. Whether drug-induced perseveration shares neural processes responsible for drug-induce reinforcement remains to be determined. (Supported by NSERC).

470.1

RESPONSES OF NEURONS IN THE RAT NUCLEUS SUBMEDIUS TO NOXIOUS AND INNOCUOUS MECHANICAL CUTANEOUS STIMULATION.
J.A. Coffield* and V. Miletic (SPON: A. Messing). Dept. Comp. Biosci., Sch. Vet. Med., Univ. of Wisconsin, Madison, WI 53706.

Extracellular recordings were used to examine the responses of 78 neurons in the rat nucleus submedius (SM). Responses of 13/18 cells activated only by noxious stimuli were of swift onset and rapid termination. In contrast, responses of 3 cells were delayed both in onset and termination, and in 2 neurons the response was immediate, but the increased evoked activity outlasted stimulus application by 13 min. Receptive fields (RFs) of these nociceptive neurons were generally large, although none were bilateral. Four SM neurons were activated by innocuous stimuli, but their maximal response was obtained only after noxious stimulation. Responses of all 4 neurons were immediate in onset and recovery, and their RFs were large (two were bilateral). Responses of 7/12 neurons activated only by innocuous stimuli were rapid in onset and termination, while that of 3/12 were delayed in both onset and termination. Two of the 12 innocuous-only cells became unresponsive to repeated stimulation, and could be re-activated only after a period during which no stimuli were applied. RFs of these units were also generally large (three were bilateral). Five SM neurons responded by decreasing or ceasing their firing subsequent to noxious only (n=2), or innocuous only (n=3) stimulation. Four of these units had large RFs (two were bilateral). The remaining 39 SM neurons were not activated by mechanical cutaneous stimulation. Electrical stimulation of the ventrolateral orbital cortex was used to examine projections of 21/39 characterized SM neurons. Ten cells were activated synaptically, while two neurons were inhibited.

These results provide further support for the involvement of SM neurons in nociception, and additionally suggest that their role is not limited to nociceptive information signalling but encompasses a wider range of cutaneous sensations. (Supported by NS26850).

470.2

BRAINSTEM AFFERENTS AND CORTICAL PROJECTIONS OF THE RAT NUCLEUS SUBMEDIUS. J.O. Dostrovsky, A. Yoshida*, C.Y. Chiang* and B.J. Sessle. Dept. of Physiology, and Fac. of Dentistry, Univ. of Toronto, Canada.

Previous studies (Craig & Burton J. Neurophysiol. 45,443,1981, Craig et al JCN 206,28,1982) have described in the cat projections from the marginal layer of trigeminal (V) subnucleus caudalis to nucleus submedius (SM) and from SM to the ventrolateral orbital cortex (VLO). Dostrovsky & Guilbaud (Brain Res. 460,269,1988) have recently reported that neurons in rat SM respond to nociceptive inputs, but the anatomical connections of SM in the rat have not been characterized in detail. Iontophoretic injections of wheat-germ agglutinin horseradish peroxidase were made in the rat SM or VLO. Following a survival period of 3-4 days the animals were perfused and tissue sectioned and processed with the TMB reaction. Following SM injection dense anterograde labeling was observed in the ipsilateral VLO and adjacent lateral orbital cortex. A small number of retrogradely labeled neurons were observed in both ipsilateral and contralateral VLO and a small dense cluster in the ventromedial bank of the ipsilateral prefrontal cortex. Following injections into VLO dense retrograde and anterograde labeling was observed in SM. Some retrogradely labeled cells were also observed ipsilaterally in mediodorsal thalamic nucleus and contralaterally in VLO. Following SM injections many contralaterally and some ipsilaterally labeled neurons were observed in the caudal part of V subnucleus interpolaris and a few in caudalis. These findings confirm previous reports of connections between SM and prefrontal cortex and the V nucleus and SM. Despite many similarities between these pathways in the rat and cat, a notable difference was the sparse projection from the marginal layer of V subnucleus caudalis in the rat. Supported by NIH DE05404 and Canadian MRC.

470.3

FUNCTIONAL RELATIONSHIPS AMONG NUCLEUS GIGANTOCELLULARIS, DORSAL CENTRAL GRAY, AND PARAFASCICULARIS NUCLEUS WITH REGARD TO NOCICEPTION AND RELATED BEHAVIORS IN RATS. V.J. Roberts*. (SPON: W.K. Dong). Dept. of Anesth., Univ. of Washington Sch. of Med., Seattle, WA 98195

The functional properties of the nucleus gigantocellularis (NGC) were investigated by examining behavioral responses that are supported by NGC stimulation in rats. Analyses indicated that NGC stimulation will support escape, active avoidance learning, increases in behaviors that are thought to reflect fear in rats, and a variety of elicited spinal somatic and orofacial movements. These results provide behavioral evidence for NGC involvement in several aspects of nociception. Alterations in responses following lesions of the dorsal central gray (DCG) or the parafascicularis (PF) were examined in the same group of rats. Lesions of the DCG decreased affective responses and facilitated extinction of avoidance responses associated with NGC stimulation. Lesions of the PF disrupted escape and avoidance, although postlesion training improved avoidance but not escape performance. These results were interpreted in terms of a general role for the DCG in aversive affective components of nociception, and for the PF, in execution of behavioral/motor responses to nociceptive stimuli. The finding that the elicited responses were unaltered following either lesion is consistent with a role for the NGC in motor aspects of nociception, and in the relay of this information to rostral neural structures.

470.4

ANATOMIC QUANTITATION OF POTENTIAL NOCICEPTIVE INPUT TO THE PRIMARY SOMATOSENSORY CORTEX (S1), S.I. GINGOLD*, J.D. GREENSPAN, and A.V. APKARIAN, Dept. of Neurosurg., SUNY Hlt. Sci. Ctr., Syracuse, NY 13210.

Nociceptive input to S1 was estimated by counting possible contacts between spinothalamic terminals (STT-t) and thalamocortical cells (TC-c). Monkeys were injected with fluorescent dyes in the hand areas of S1, and with HRP in the contralateral cervical enlargement. Labeled terminals and cells were mapped in the thalamus, and their overlap determined by counting the number of TC-c which fell within a given distance from STT-t: somatic hit distance (SH) was a 25μ radius, proximal dendritic hit (PDH) was 50μ, and distal dendritic hit (DDH) was 100μ.

With complete S1 hand area injections, TC-c were primarily found in four thalamic nuclei: ventroposterior lateral, VPL (n≈2000/100μ); anterior pulvinar, Pa; centrolateral, CL (n≈200/100μ); and ventroposterior superior, VPS. CL had the largest percentage of overlap (%SH=12-18; %PDH=22-30; %DDH=36-45), while both VPS and Pa had the lowest percentage of overlap (%SH≈3, %PDH≈6, %DDH≈15). In VPL, TC-c were in the forelimb region and STT-t were usually lateral and/or dorsal to the TC-c (%SH≈8; %PDH≈15; %DDH≈27). The overlap of 3a, 3b, and 1 projecting TC-c was also compared directly by injecting 3 fluorescent dyes in these regions in the same animal.

These results reveal that a small fraction of S1 TC-c can receive direct STT-t input, but individual thalamic nuclei have distinct input-output patterns.

470.5

THE VALIDITY OF OPERANT RESPONSE MAGNITUDE AS A PAIN INTENSITY MEASUREMENT. D.K. Douglass*, I.G. Campbell*, E. Carstens, and L.R. Watkins. Dept. of Animal Physiology, U.C. Davis, Davis, CA 95616.

Cooper and Vierck have conducted monkey studies which suggest that the magnitude of an operant response which terminates a noxious electrical stimulus is correlated with the intensity of the stimulus. The present experiment examines the reliability of operant response magnitude as an indicator of intensity of pain induced by radiant heat. In 8 human subjects, the force with which subjects pressed a button to terminate a noxious stimulus was compared to pain intensity ratings on visual analog scales. 10 radiant heat stimuli ranging from 40 to 54^{o}C were applied to the dorsal forearm in a pseudo-random order at nine separate stimulation sites. The stimulus automatically terminated when the subject pressed the termination button or following a maximum duration of 3 secs. (2 secs. for 54^{o}C). In one study subjects were instructed to press the termination button whenever they wished to terminate the stimulus; in another study, the subjects pressed the button when they first detected pain. In both the termination and detection studies,the correlation between stimulus temperature and operant response force was very poor (R=0.161, R=0.367 respectively). In the detection study, the operant response latency did correlated (R=0.856) with the stimulus temperature. In both the termination and detection studies, pain intensity was not correlate (R=-0.067, R= 0.281 respectively) with operant response magnitude. There was a slight correlation between operant response latency and pain intensity (R=0.531) in the detection study. The data indicate that operant response magnitude is not a valid indicator of pain intensity, but that operant response latency may, after further development, prove to be a reliable indicator. Also, the current data indicate that the magnitude of an operant response which terminates a noxious stimulus does not depend on the magnitude of the stimulus. Supported by NIH NS20037.

470.6

THE VALIDITY OF WITHDRAWAL MAGNITUDE AS A PAIN INTENSITY MEASUREMENT. I.G. Campbell*, E. Carstens, D.K. Douglass*, L.R. Watkins (Spon: R. Kitchell). Dept. of Animal Physiology, U.C. Davis, Davis, CA 95616

Previous experiments have demonstrated that, in lightly anesthetized rats, the magnitude of withdrawal from a noxious radiant heat stimulus is correlated with the intensity of the stimulus. The present experiment examines the reliability of the withdrawal magnitude as an indicator of the intensity of pain induced by radiant heat. In 10 healthy humans the magnitude of forearm flexion withdrawal as measured by biceps EMG recordings was compared to pain ratings on visual analog scales. 10 heat stimuli ranging from 40 to 54^{o}C were applied to the dorsal forearm in a pseudo-random order at nine separate stimulation sites. The stimulus automatically terminated upon arm withdrawal or at a maximum duration of 3 secs., 2 secs. for 54^{o}C. The stimulus response functions for mean withdrawal magnitude (correlation coefficient R=0.989), mean inverse of withdrawal latency (R=0.998), and mean pain intensity (0.969) were all positively accelerating functions. Above 48^{o}C the pain intensity function leveled off, while the withdrawal magnitude and inverse of latency continued to increase exponentially. A log-log scatter plot of each subject's pain intensity ratings of each stimulus vs. the magnitude of withdrawal evoked by the same stimulus, indicates that there was little correlation (R=0.434) between withdrawal magnitude and pain intensity. The correlation was equally poor (R=0.427) for a similar plot of pain intensity vs. the inverse of withdrawal latency. Although the mean stimulus response functions for pain intensity and withdrawal magnitude or withdrawal latency are similar positively accelerating functions at lower temperatures, the poor correlation between withdrawal magnitude and pain intensity indicates that withdrawal magnitude may not be a reliable indicator of pain perception. Supported by NIH NS20037.

470.7

SOLITARY NUCLEUS AND SPINAL CORD INPUTS TO THE LATERAL PARABRACHIAL (PBNl) NUCLEUS IN CAT. S. Scofield* and K. J. Berkley (SPON: M. Berkley). Dept. of Psychology, Florida State Univ., Tallahassee, FL 32306.

The PBN is known to relay visceral information from caudal parts of the solitary nucleus and cutaneous nociceptive information from the spinal cord to the forebrain. The present study used double anterograde tracing methods to compare the distribution of these two inputs within PBNl. While spinal input was centered dorsally and solitary input ventrally in PBNl, the inputs overlapped substantially at their adjacent borders. Comparison with previous work indicated that the forebrain target of neurons in the overlap zone was mainly the hypothalamus, whereas the target of neurons in the non-overlap zones was mainly thalamus (spinal cord) or amygdala (solitary nucleus). These results suggest that functional distinctions between solitary-visceral and spinal-cutaneous inputs are likely to be maintained in PBNl's outputs to some forebrain targets, but mutually modified in its output to the hypothalamus. The results also support PBN's putative complex roles in autonomic and pain regulation.

Supported by NIH grant NS 11892.

470.8

Anatomical evidence for calcitonin gene-related peptide and dynorphin A inputs on to spinomesencephalic tract neurons in the rat spinal cord. R. L. Nahin, E. Humphrey and J.L.K. Hylden. Neurobiology and Anesthesiology Branch, NIDR, NIH. Bethesda MD, 20892.

Calcitonin gene-related peptide (CGRP) is associated with small diameter nociceptive primary afferent fibers in the spinal cord. The opioid peptide dynorphin (DYN) is found within both neuronal cell bodies and terminals in the spinal cord. The present study addressed whether these two peptides, which are associated with nociception, are contained within terminals making direct contacts on to a population of known nociceptive neurons, lamina I spinomesencephalic tract (SMT) neurons. Animals were injected with retrograde tracer into the caudal midbrain. Two different tracers were used: the inactive B-subunit of cholera toxin (CT), or wheat germ agglutinin conjugated to horseradish peroxidase (WGA-HRP). WGA-HRP was visualized with a modified TMB protocol in which the reaction product was stabilized by post-incubation of the tissue in DAB. CT was visualized immunocytochemically, using an antisera developed against cholera toxin. Tissue was also processed for either DYN or CGRP immunoreactivity. 167 retrogradely labeled SMT lamina I neurons were examined and classified according to their morphological type. Of these, 58 had CGRP puncta in close apposition to either their soma or proximal dendrites (4.2±2.8 contacts/cell). No correlation was seen between morphological type and frequency of CGRP contacts; a positive correlation was seen between cell size and the number of contacts. Preliminary findings also indicated that retrogradely labeled neurons in laminae I, V-VIII and X receive DYN contacts. These data suggest that a population of supraspinally projecting nociceptive neurons in lamina I receive both small diameter primary afferent input and input from opioid neurons thought to be involved in the local modulation of nociception.

470.9

TERMINATIONS OF LAMINA I SPINOPARABRACHIAL PROJECTIONS. IN THE RAT. R.M.Slugg and A.R.Light. Dept. of Physiology, UNC-Chapel Hill, Chapel Hill, NC 27599-7545.

The projection of spinal cord lamina I neurons to sites within and near the pontine parabrachial nucleus has been documented by anterograde and retrograde studies in the rat, cat, and primate. The present study was designed to analyze the "Golgi-like" immunohistochemistry in fibers and terminals anterogradely labeled by phaseolus vulgaris leucoagglutinin (PHA-L) from discrete iontophoretic injections confined to either the dorsal horn or lamina I in the rat. Immunoreactive fibers from the spinal cord ascended from the ventral lateral pons and coursed with the ventral spinocerebellar tract prior to entering the parabrachial nucleus. PHA-L immunoreactive fibers of fine caliber with en passant and terminal boutons were found primarily in the rostral part of the lateral parabrachial nucleus contralateral to the injection site. Single fibers with several branches and numerous boutons were observed to cover large portions of the lateral parabrachial nucleus dorsal to the lateral pole of the superior cerebellar peduncle. Fibers with boutons were also observed in the medial parabrachial nucleus, nucleus Kolliker-Fuse, and within the superior cerebellar peduncle. Fibers with a lower density of boutons were observed rostrally in the nucleus cuneiformis. Projections to Kolliker-Fuse were heaviest when the injection site was not limited to lamina I. Supported by grants PHS# DA04420 and NS16433.

470.10

COLLATERALIZATION OF SPINOMESENCEPHALIC TRACT (SMT) AXONS. C.M. Mendez*, J.G. Broton, R.P. Yezierski. Dept. of Neurological Surgery, Univ. of Miami Sch. of Med., Miami, FL 33136.

The SMT is a multicomponent pathway that has been shown to have varied origins, spinal trajectories and sites of termination. Cells belonging to this pathway respond to noxious mechanical and thermal stimuli as well as input from muscles, joints and visceral structures. Because of this the SMT is thought to have a role in sensory, motor and visceral functions. In the present study anatomical and physiological techniques were used to evaluate the spinal and supraspinal collateralization of SMT axons. Using the retrograde transport of different tracers, experiments were carried out in the rat to identify: (a) cells with bilateral projections to midbrain; and (b) cells with ascending projections to midbrain and descending propriospinal projections. The supraspinal collateralization of identified SMT axons in the cat was studied using antidromic stimulation to map the trajectory of axons and collaterals through the midbrain. The results of these experiments have shown that some SMT cells have divergent projections to multiple levels of the midbrain or to midbrain and spinal cord. Cells in the latter population were found in the marginal zone, the lateral cervical and spinal nuclei, the lateral neck of the dorsal horn and in the region around the central canal. Bilaterally projecting cells were found in these regions and additionally in nucleus proprius. The results of antidromic mapping experiments have shown that some SMT axons can be backfired from multiple low threshold sites at different levels of the midbrain and from sites in midbrain and thalamus.

Supported by funds from NS19509 and the Miami Project Foundation.

470.11

ANTAGONISTIC EFFECTS OF LESIONS IN ANTEROLATERAL COLUMNS (ALC) AND DORSOLATERAL FUNICULI (DLF) ON REACTIONS TO ACUTE AND CHRONIC NOXIOUS STIMULI IN RATS. N.E. Saadé, S.F. Atweh and S.J. Jabbur. Fac. of Med., American University of Beirut, Beirut, Lebanon.

The possible role of the ALC and DLF in pain mechanisms were examined from the effects of lesions in these tracts on tests of acute pain (hot plate-HP, and tail flick-TF) and chronic deafferentation pain (autotomy-AT). Spinal lesions (C_{2-3}) were performed on rats under anesthesia. In the first set of experiments, AT scores were compared in subgroups with either ALC lesion followed by contralateral leg denervation (denv) (n=9), or sham surgery+denv (n=9) or denv only (n=9), and showed significant decrease in AT in ALC lesioned subgroup. In the second set of experiments, AT scores and HP-TF latencies were compared during 7 weeks after surgery in the following subgroups: ALC lesion+denv (n=17), ALC lesion without denv (n=6), DLF lesion+denv (n=6), DLF lesion without denv (n=6) and sham operated (n=6). DLF lesion with or without denv caused no change in HP and TF, but subgroup with denv showed increased AT. HP and TF latencies increased after ALC lesion and showed further increase in ALC lesion+denv during 2-3 weeks following denv. Results suggest that in ALC lesioned rats+denv, reduction of chronic pain (AT) is due to continuous triggering of descending inhibitory mechanisms through intact DLF as revealed by persistent increase in HP and TF latencies. (Supported by LNRC and DTSabbagh grants).

470.12

EFFECTS OF VARIOUS SPINAL LESIONS ON AUTOTOMY (AT) INDUCED BY BILATERAL LIMB DENERVATION IN RATS. S.J. Jabbur, L. Shihabuddin, S.F. Atweh and N.E. Saadé. Fac. of Med., American University of Beirut, Beirut, Lebanon.

Wall et al. (Pain, 35:327, 1988) reported differential effects of dorsolateral funiculus (DLF) and dorsal column (DC) lesions on AT following one leg denervation (denv) made either simultaneous with or few days after spinal lesions. The present study examined the effects of various chronic spinal lesions on AT induced by bilateral hind limb denv. Under anesthesia, one sided spinal (C_{2-3}) lesions were made at the following sites: DLF (n=8), dorsal quadrant (DQ) i.e., DC+DLF (n=9), anterolateral columns (ALC) (n=6) and hemisection (n=8). After a 5 weeks period, all rats had bilateral section of sciatic and saphenous nerves and percentage of occurrence, score, onset and duration of AT were observed. Lowest percentages (≤50%) and scores (≤4) occurred in rats with ALC lesions, to be followed by rats with hemisection and highest percentages (100%) and scores (≥10) occurred in rats with DQ, to be closely followed by rats with DLF lesions. Similar AT patterns were observed in both legs. Our results suggest that spinal and supraspinal mechanisms play an important role in AT which is thus related to pain and influenced by lesions of ascending nociceptive-carrying and descending nociceptive-controlling mechanisms. (Supported by LNRC and DTSabbagh grants).

470.13

AUTOTOMY (AT) IN RATS IS PRODUCED BY CHRONIC SPINAL LESIONS ONLY IF PRECEDED BY PREVIOUS EXPOSURE TO PAIN. S.F. Atweh, N.E. Saadé, N. Wehbe and S.J. Jabbur. Fac. of Med., American University of Beirut, Beirut, Lebanon.

The report of AT in monkeys following selective spinal lesions (Levitt and Levitt, Pain, 10:129, 1981) had been correlated with recurrence of pain following human anterolateral cordotomy. This study examined whether spinal lesions (without denervation) could induce AT in rats and whether this could be altered by previous injury. In the first set of experiments, one-sided spinal (C_{2-3}) lesions were performed under anesthesia at indicated sites in following subgroups: hemisection (n=8), anterolateral columns (ALC)(n=26), dorsolateral funiculus (DLF)(n=13), dorsal columns (DC)(n=5) and DLF+DC (n=9). No AT was reported during 5-10 weeks of observations. Hemisected and ALC lesioned rats showed some increase in pain threshold. In the second set, similar lesions (hemisection n=6, DLF n=6, ALC n=6 and DLF+DC n=6) were preceded at 3-6 hrs by s.c. injection of 0.1 ml of 3% formalin in dorsal aspect of both legs. During 5 weeks of observations, mild signs of AT were observed in about half the rats in ALC and hemisected subgroups. These results suggest a role of previous injury in triggering AT and may be related to the recurrence of pain after cordotomy in patients with long history of intractable pain. (Supported by LNCR and DTSabbagh grants).

470.14

ASCENDING LAMINA I AXONS IN THE CAT ARE CONCENTRATED IN THE MIDDLE OF THE LATERAL FUNICULUS. A. D. Craig, Jr. Divisions of Neurobiology and Neurosurgery, Barrow Neurological Institute, Phoenix, AZ 85013.

The ascending axons of specifically nociceptive and thermoreceptive lamina I neurons form half of the spinothalamic tract (STT) in the cat, but their role in pain and temperature sensation has been challenged by reports that they ascend in the dorsolateral funiculus. Direct observations have been made following injections of the anterograde tracer PHA-L in lamina I at different spinal segments. Ascending lamina I axons are variably located throughout the contralateral white matter, but in general are concentrated in the middle of the lateral funiculus, i.e., at the level of the central canal. This finding is consistent with the location of lesions affecting thermoreception in cat and, given the increase in size of the corticospinal tract in man, with several descriptions of anterolateral chordotomies. The retrograde labeling data reported earlier by others may have been complicated by the transneuronal retrograde transport of WGA*HRP, its weak efficacy for labeling lamI-STT neurons, and the inherent variability in the location of ascending lamina I axons.
(Supported by NS 25616 and the Barrow Neurological Foundation.)

470.15

THE ROLE OF CORTICAL, THALAMIC, AND SUBTHALAMIC NEURONS IN PAIN: EVIDENCE FROM LASER EVOKED POTENTIALS AND SENSORY TESTING IN PATIENTS AND NORMAL SUBJECTS. K.L. Casey, G. C.-H. Huang and T. J. Morrow, Depts. of Neurology and Physiology, Univ. of Mich. and Neurology Research, V. A. Med. Ctr., Ann Arbor, MI 48105.

Cutaneous stimulation with infra-red lasers selectively activates small diameter afferent fibers and generates cerebral potentials that vary in amplitude with pain intensity (Carmon et al., 1978). We recorded averaged evoked potentials from the vertex of 10 normal volunteers and 7 patients whose hands were stimulated with infra-red laser beams at pricking pain threshold intensities (ave. 10W, 60msec, 9mm diam., 50 trials). All normal subjects generated a negative - positive potential (av: 11.4uV pk-pk, 235msec first peak). Four patients with unilateral radiologically identified subthalamic lesions (1 syrinx, 3 brainstem infarctions) had unobtainable deep pain (pressure algometer to bone) and thermal pain thresholds (contact thermode) contralaterally; vibration, graphesthesia, 2 PD and kinesthesis were normal. They generated normal vertex potentials with ipsilateral stimuli, but contralateral stimulation evoked either nothing or potentials that were attenuated (5uV pk-pk) and delayed (550msec). Two patients with focal posterior lateral thalamic lesions (MS and lacunar stroke) had contralaterally elevated thermal pain thresholds (51°C), but normal deep pain and mildly reduced vibratory sense; contralateral laser stimulation evoked attenuated potentials (2 - 3uV pk-pk). One patient with a stroke sparing the thalamus but involving frontal, parietal and occipital cortex, had normal thermal and deep pain thresholds but markedly impaired tactile discriminative and kinesthetic sense. Laser pulses to the abnormal hand evoked only a 2uV positive potential at 550msec. **These results suggest that: 1) laser-evoked potentials are generated by thalamo-cortical neurons that are selectively activated by noxious stimuli; 2) these neurons are not necessary for pain; 3) pain is mediated primarily by subcortical neuronal systems.** (Supported by Dept. of V.A. and a Bristol-Myers award).

470.16

SPECTRAL EEG CHANGES WITH COLD PRESSOR. **M.Backonja*, J.Wang*, E.W.Howland*, J.Smith*, C.S. Cleeland*(SPON: H. Schutta). Pain Research Group, Dept. of Neurology, Univ. of Wisc. Madison, WI. 53792.**

Although severe pain in humans, especially chronic pain has profound effect on affective and cognitive suprathalamic processes, physiologic data for changes in these processes are slight.

Spectral analysis of EEG of 4 healthy subjects was recorded during immersion of the right hand in painful cold water (0-2°C) and during a nonpainful cool water (20°C) control. Averages of the spectrum from 13 artifact free epochs (five seconds each) were used to calculate an asymmetry ratio (right-left/right+left) for 6 pairs of electrodes. Repeated measures analysis of variance of these asymmetry ratios for 4 subjects revealed significant effects for electrode and electrode by pain effects in the alpha band but not in the delta or theta bands. Post-hock comparisons revealed that only the posterior electrode pairs (P3-P4, T5-T6) showed significantly higher asymmetry ratios during cold pressor than during control stimulation. These ratios reflect relatively greater right sided alpha power. Physiologic implications will be discussed in the presentation.

470.17

PHYSIOLOGY OF CELLS WITH NOCICEPTIVE INPUT IN THE GRACILE NUCLEUS OF CATS. K.D. Cliffer, T. Hasegawa* and W.D. Willis, Jr., Marine Biomedical Institute, University of Texas Medical Branch, Galveston, TX 77550

Unit recordings were made in the gracile nucleus of cats, anesthetized with chloralose and pentobarbital, and paralyzed with Flaxedil. Discharges of 65 cells were found and isolated on the basis of responses to varied mechanical stimuli. Of these, 22% responded to noxious heat, either initially or after sensitization, indicating input originating in nociceptors. These also had low-threshold mechanical input and varied levels of response to noxious mechanical stimuli. Most also responded to stretching of the skin. Receptive fields were usually moderate in size, comprised of part of the ipsilateral hindlimb. These cells were scattered widely in the nucleus from 4 mm caudal to the obex to 0.6 mm rostral to it. We were able to activate 36% of the nociceptive cells and 25% of all recorded cells from the thalamus. For some, including several with nociceptive input, the most rostral points from which they could be activated with low currents (<25μA) was determined and considered to be near the most rostral projections of the axons in the ventrobasal complex. Our results indicate a greater and more widespread population of neurons in the cat gracile nucleus with nociceptive input, many with projections to the thalamus, than has been previously appreciated. [Supported by grants from NIH (NS 11255, NS 09743 and postdoctoral fellowship NS 08151 to K.D.C.) and the Bristol-Myers Co.]

470.18

JOINT AND MUSCLE A- AND C-AFFERENT FIBER CONVERGENCE IN RAT TRIGEMINAL (V) BRAINSTEM NEURONS. J.W. Hu*, Y. Sharav* and B.J. Sessle. Faculty of Dentistry, University of Toronto, Toronto, Ontario, M5G 1G6, Canada.

The present study was initiated to examine possible deep afferent inputs from temporomandibular joint (TMJ) and hypoglossal nerve (XII) muscle afferents to the rat V subnucleus caudalis (medullary dorsal horn). Extracellular single neuron recordings were made from caudalis of 14 rats anesthetized with urethane-chloralose. Neurons were classified on the basis of their cutaneous mechano-receptive field properties as low-threshold mechano-receptive (LTM, n=83), wide dynamic range (WDR, n=14) and nociceptive-specific (NS, n=27). TMJ or XII electrical stimulation excited respectively 8% and 0% of the LTM neurons, 64% and 50% of the WDR neurons and 15% and 4% of the NS neurons. Latencies of responses to TMJ stimulation were longer than those to skin electrical stimulation but shorter than those to XII stimuli. 50% of the WDR neurons had A and C fiber inputs evoked by high-intensity (2ms, 5 mA) stimulation of TMJ and XII as well as skin, whereas only 1 NS neuron and no LTM neuron had a C as well as A fiber TMJ or XII input. These results provide important insights into the organization of deep inputs from TMJ and masticatory muscles to central V nociceptive pathways and suggest that the demonstrated convergent mechanisms in caudalis may play a role in mechanisms of deep pain in the orofacial region. (Supported by NIDR grant DE-04786).

470.19

THE ULTRASTRUCTURE OF TRIGEMINAL VASCULAR CONVERGENCE NEURONS. S. Potrebic*, A. Strassman, E.A. Hartwieg*, R. Maciewicz. Pain Physiology Laboratory, Mass General Hospital, Boston MA 02114.

Brainstem trigeminal vascular convergence (TVC) neurons receive an excitatory, nociceptive input from cranial blood vessels as well as facial skin or cornea. TVC cells may mediate the pain associated with vascular headache. In the present study, cat TVC neurons were electrophysiologically identified and then intracellularly labelled with horseradish peroxidase. One population of TVC neurons has axons which collateralize extensively in the trigeminal complex. Representative neurons of this class were examined with electron microscopy. The labelled TVC neurons are located in ventrolateral Lamina IV and V of trigeminal nucleus caudalis(TNC). The cells have myelinated axons and collaterals that give rise to unmyelinated preterminal processes. Within Lamina IV and V of TNC, terminals of TVC cells contain round synaptic vesicles and form synapses on dendrites, spines, and, less frequently, on cell somas. Based on these morphological features, we hypothesize that at least some TVC cells which collateralize in the trigeminal complex provide excitatory input to other trigeminal neurons. These cells may play a role in the cutaneous hyperalgesia associated with vascular head pain.

RESPIRATORY REGULATION II

471.1

IN VITRO CONTRACTILE PROPERTIES OF MOTOR UNITS IN ADULT HAMSTER DIAPHRAGM. M. Fournier and G.C. Sieck. Department of Biomedical Engineering, University of Southern California, Los Angeles, CA 90089.

The physiological properties of diaphragm (DIA) motor units (MU) were studied using an *in vitro* preparation. While perfusing the animal with oxygenated Ringer's, the right hemidiaphragm was dissected in continuity with the phrenic nerve and its cervical ventral roots. Thereafter, the muscle-nerve-ventral root preparation was placed in a temperature-controlled (27°C) chamber continuously perfused with oxygenated Ringer's. Stimulation of ventral roots C_3 to C_5 indicated somatotopy in DIA innervation. Motor units were isolated by dissection and graded stimulation (using a suction electrode) of ventral root filaments. Based on "sag" and a fatigue test, MU were classified into four types. A wide range in contractile properties were observed with slow-twitch units generating the lowest tensions. Twitch tensions of MU ranged from 15 *mg* to 1.2 *g*. Twitch contraction times ranged from 24 to 90 *msec* and half-relaxation times from 34 to 160 *msec*. Maximum tetanic tensions of MU ranged from 96 *mg* to 3.3 *g* compared to an aggregate maximum tension of ~120 *g* for the entire costal region. These results indicate that the adult hamster DIA is comprised of MU varying considerably in their physiological properties. Furthermore, the *in vitro* preparation provides a valuable controlled enviroment in which to study MU properties.

(Supported by NIH grants HL34817 and HL37680.)

471.2

COMPARISON OF FIBER SUBTYPE DISTRIBUTION OF PHARYNGEAL DILATOR MUSCLES AND DIAPHRAGM IN CAT. E. van Lunteren and T.E. Dick. Department of Medicine, Case Western Reserve University, Cleveland, OH, 44106.

Differences exist between the pharyngeal dilator muscles and the thoracic respiratory muscles in their patterns of electrical and mechanical activity during the respiratory cycle, both in normal humans and animals and in subjects with sleep-related ventilatory disorders such as obstructive sleep apnea. Little is known about the intrinsic properties of the pharyngeal muscles, however, and how they relate to the intrinsic properties of the diaphragm. In the present study, the fiber subtype distributions of two pharyngeal dilator muscles, the geniohyoid and sternohyoid, were ascertained histochemically in the adult cat. Muscle was removed under anesthesia (n=5 for hyoid muscles, n=7 for diaphragm), rapidly frozen in liquid nitrogen, sectioned, and stained for myosin ATPase (alkaline pH) and NADH tetrazolium reductase. Both the geniohyoid and sternohyoid muscles had a preponderance of fast glycotylic (FG) fibers (48% and 55%, respectively), a smaller number of fast oxidative glycolytic (FOG) fibers (36% and 31%, respectively), and few slow oxidative (SO) fibers (16% and 14%, respectively). Both the geniohyoid and sternohyoid had lower proportions of SO fibers than the diaphragm ($P < 0.001$ and $P < 0.001$, respectively), and significantly greater proportions of FOG fibers and FG fibers than the diaphragm. These data indicate that the geniohyoid and sternohyoid muscles have histochemical characteristics associated with fast contraction times but low endurance properties. We speculate that the pharyngeal dilator muscles may not be well suited to the continued activation required when upper airway patency is comprised by the structural changes that often are present in subjects with sleep apnea. SUPPORT: HL-38701, HL-01600.

471.3

DETECTION AND CORRECTION OF EMG INTERFERENCE DURING NERVE CUFF RECORDING. E.M. Ebly, C.L. Cleland, J. Brandt*, P. Getting and J. Maloney.*. Dept. Obst. and Gynecol., University of Calgary, Calgary, AB, Canada T2N 4N1 and Dept. Physiol. and Biophys., University of Iowa, Iowa City, IA 52242

Chronic recording from nerves using cuff electrodes is common in research and has prosthetic applications. However, the small neural signal can be swamped by the electrical activity of nearby muscles (EMG). Current techniques for suppressing EMG, such as tripolar recording and filtering, can be inadequate. Thus, we developed a technique in which a second set of electrodes on the external surface of the cuff, which record *only* EMG, can be used to *detect* or *correct* for EMG contamination of the neural signal.

Detection: Tripolar cuff electrodes, with both internal and external electrodes, were implanted on the vagus nerve in lambs. The signal recorded by the external electrodes proved a useful indicator of when neural signals were contaminated by EMG.

Correction: If EMG recorded by the external and internal electrodes were identical, then subtraction would eliminate EMG from the internal signal. This hypothesis was tested using a bipolar electrode in a saline bath and a moveable electrode dipole as the EMG source. We found that the external and internal signals were similar only when an additional tube was placed around the cuff, producing similar current flow across the internal and external electrodes. Thus, subtraction of the external from the internal signal can attenuate interference from nearby muscles and other external current sources.

Funded by Alberta Heritage Foundation for Med. Res. and NIH.

471.4

INTERSEGMENTAL EXCITATION OF CERVICAL INSPIRATORY NEURONS BY LOWER INTERCOSTAL NERVE AFFERENTS. R. Shannon, Y.M. Hernandez*, and B.G. Lindsey. Dept. Physiol. & Biophysics, Col. Med., Univ. South Florida, Tampa, FL 33612

We previously reported (Soc. Neurosci. Abst. 14:626) that 40% of the inspiratory cells located in the cervical spinal cord (C1-2) of anesthetized cats were excited by lower (T9-10) intercostal nerve afferents (Ib from tendon organs) through intersegmental pathways. We conducted experiments to determine if the lack of an excitatory response in some I-cells resulted from anesthetic depression of the reflex. Studies were performed on 4 unanesthetized mid-collicular decerebrate cats that were thoracotomized, paralyzed and ventilated. Extracellular cervical neuron and phrenic (C5) efferent activities were recorded. Electrical stimulation of low threshold T9-11 internal intercostal nerve afferents, which excited phrenic activity through intersegmental pathways, elicited an excitatory response in only 2 of 38 (5%) I-cells. All cells and the phrenic decreased activity following the excitatory phase in the phrenic.

These results do not resolve whether anesthetics were responsible for the lack of an excitatory response in some I-cells in anesthetized cats. The results suggest that suprapontine structures participate in the gating of the excitatory effects of lower intercostal tendon organ afferents on cervical I-cells. (Supported by USF Research and Creative Scholarship Grant)

471.5

POSSIBLE INHIBITION OF PHRENIC MOTONEURONS AND MEDULLARY INSPIRATORY NEURONS BY BOTZINGER EXPIRATORY NEURONS DURING (FICTIVE) VOMITING. A.D. Miller and S. Nonaka*. Rockefeller Univ., New York NY 10021

During vomiting, the diaphragm and abdominal muscles co-contract in a characteristic series of bursts of activity. Bulbospinal expiratory (E) neurons in the caudal ventral respiratory group fire appropriately to activate E spinal motoneurons during fictive vomiting (FV) (1). In contrast, most bulbospinal inspiratory (I) neurons in the dorsal and ventral respiratory groups could not initiate activation of the diaphragm during FV, because either the neurons are silent or fire near the end of phrenic discharge (2).

We now report on the behavior during FV of Botzinger (BOT) E neurons, which make inhibitory connections with medullary I neurons and phrenic motoneurons (3). FV was elicited in decerebrate, paralyzed cats by emetic drugs or electrical stimulation of abdominal vagal afferents. BOT E neurons, antidromically activated from C4, fired between bursts of phrenic discharge during FV and thus could at least partly account for the concurrent lack of firing of these motoneurons. These BOT E neurons may also inhibit bulbospinal I neurons during FV, but this input by itself is insufficient to account for longer duration silencing of bulbospinal I neurons during FV. Supported by NIH grant NS20585.

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471.6

FLUORESCENT MICROBEAD LOCALIZATION OF ROSTRAL VENTROLATERAL MEDULLA (RVLM) PIRENZEPINE AND 4-DAMP MICROINJECTIONS THAT DECREASE BASELINE AND CO2 SENSITIVE PHRENIC OUTPUT. Eugene E. Nattie and Ainua Li*. Department of Physiology, Dartmouth Medical School, Hanover, N.H. 03756.

Application of the M1 muscarinic receptor antagonist pirenzepine but not the M2 cardiac receptor antagonist AF-DX 116 to the RVLM surface by cotton pledgets in chloralose urethane anesthetized, paralyzed, deafferented, servoventilated cats decreases CO2 sensitivity (J. Appl. Physiol 66:1462-1470, 1989). This study microinjects (10 nl) pirenzepine or the M2 glandular receptor subtype antagonist 4-DAMP mixed with fluorescent microbeads to 1) ask if the pirenzepine effect could involve M2 glandular receptors, and 2) localize the anatomical site of the effects. Microinjections of pirenzepine or 4-DAMP within 300 um of the RVLM surface in a region between the superior and inferior olives just below the facial nucleus decreased baseline phrenic output and CO2 sensitivity. More caudal sites had smaller or no effects. We conclude that muscarinic receptor involvement in central chemoreception near the RVLM surface may be specific for the M2 glandular receptor subtype antagonized by 4-DAMP as pirenzepine can also antagonize this receptor. The location appears similar to that described for kainate microinjection induced decreases in phrenic activity and CO2 sensitivity. (Supported by HL 28066).

471.7

MEDIUM-FREQUENCY SPECTRAL PEAKS OF PHRENIC MOTONEURON AND NERVE ACTIVITIES. C.N. Christakos, M.I. Cohen, R. Barnhardt* and C.F. Shaw*. Physiol. Dept., Albert Einstein Col. Med., Bronx, New York.

The discharges of phrenic (PHR) motoneurons (MNs) and whole nerves, recorded in decerebrate paralyzed cats, were analyzed for the entire inspiratory (I) phase and for portions of I. The autospectrum of each MN's firing had a prominent component in the band of the medium-frequency spectral peak of the PHR neurogram (MFO, range 20-50 Hz), and sometimes an additional high-frequency component (HFO, range 50-100 Hz). The MFO coherence between a MN and the nerve was weak or often zero, indicating partial MFO correlations within the population. The frequency of the MN MFO component, like that of the nerve MFO peak, increased in the course of I; and it was very close to the peak discharge rate of the MN within the I portion analyzed. This correspondence between frequency of MFO spectral component and most common interval was also indicated by interval histograms of MNs (which in the presence of strong HFO could show multiple HFO-related peaks). Thus, MFO spectral peaks of PHR MNs reflect the rhythmic and augmenting discharges of the cells. These partially correlated MN rhythms give rise to an aggregate MFO in the PHR neurogram (C.N. Christakos, Intern. J. Neurosci. 29, 103-107, 1986), manifested as a broad spectral deflection with a maximum in the band of the peak firing rates of the MNs. (Supported by N.I.H. grant HL-27300.)

471.8

HIGH-FREQUENCY OSCILLATIONS (HFO) OF PHRENIC (PHR) DISCHARGE ARE ABSENT DURING FICTIVE VOMITING (VOM). M.I. Cohen, A.D. Miller, R. Barnhardt* and C.F Shaw*. Albert Einstein Col. Med. and Rockefeller Univ., New York, N.Y.

In decerebrate paralyzed cats, bilateral PHR and L1-L2 abdominal (ABD) efferent discharges were recorded during VOM produced by emetic drugs (apomorphine, lobeline, protoveratrine) or by electrical stimulation of the afferent ABD vagus (0.5 ms, 200-500 microamp pulses at 25/s). During control respiration, PHR activity had a ramp pattern and its spectrum had a large narrow peak (range 50-100 Hz in different cats), indicating presence of HFO; ABD activity had a late expiratory ramp pattern, but had no discernible spectral peak that would indicate synchronization similar to PHR HFO. During VOM, there was coactivation (a series of large-amplitude bursts having ramp patterns) of PHR and all ABD discharges. HFO spectral peaks were absent in PHR; and both PHR and ABD spectra had broad bell-shaped peaks (range 100-150 Hz). Moreover, the usual high spectral coherence (0.7-0.9) between HFOs of opposite PHRs was now absent, and the coherences between PHR and ABD discharges were zero, even though their bursts occurred together. We conclude that during VOM: a) the inputs to PHR motoneurons from the medullary inspiratory pattern generator (which produces HFO) are shut off; b) both PHR and ABD motoneurons receive inputs from another (unknown) pattern generator, but these inputs are not synchronized on a short time scale. (Supported by N.I.H. Grants HL-27300 and NS-20585.)

471.9

CARDIORESPIRATORY RESPONSES FOLLOWING MICROSTIMULATION OF SITES IN THE CAUDAL NTS OF THE RATS. L. Simpson*, M. El-Ridi*, M. Parizon* and R. Barraco. Department of Physiology, Wayne State Un. Sch. Med., Detroit, MI 48201.

Discrete bipolar electrical stimulation was administered at sites in the dorsal medulla of spontaneously breathing rats in the vicinity of the caudal nucleus tractus solitarius (NTS) and adjacent reticular formation. Cardiorespiratory responses were recorded before, during and after microstimulation to examine the functional coexistence of cardiovascular and respirtory-related neuronal elements in the NTS. Microstimulation of loci in the reticular formation adjacent to these NTS sites did not elicit any cardiorespiratory responses whereas stimulation of individual NTS regions elicited specific patterns of cardiorespiratory responses. Specifically, microstimulation of the dorsal and medial caudal NTS elicited pressor responses associated with apneic/hypopneic responses whereas stimulation of the commissural region and the ventral and ventrolateral areas of the caudal NTS elicited depressor responses associated with bradycardic and apneic/hypopneic responses. The most profound respiratory effects (ie, apnea) were seen following stimulation of the ventral and ventrolateral regions of the caudal NTS. These findings show a functional coexistence of cardiovascular and respiratory neuronal elements in the caudal NTS (supported by NSF, NIH, AHAM).

471.10

INWARD RECTIFICATION IN BULBOSPINAL NEURONS LOCATED IN THE VENTRAL PART OF THE NUCLEUS TRACTUS SOLITARIUS OF THE GUINEA PIG. M.S. Dekin, T.H. Morgan School of Biological Sciences, University of Kentucky, Lexington, KY 40506-02251

The ventral part of the nucleus tractus solitarius (vNTS) of the guinea pig comprises the dorsal respiratory group. Two classes of bulbospinal neurons have previously been identified in the vNTS (Dekin et. al., J. Neurophysiol., 58(1):195, 1987). In this report, an inward current activated by membrane hyperpolarization is described for both classes of bulbospinal neurons. All experiments were done using an in vitro brainstem slice preparation from adult guinea pigs. When voltage clamped at a membrane potential of -60 mV in the presence of 0.5 ug/ml tetrodotoxin, all neurons responded in an ohmic manner to voltage steps down to -70 mV. For voltage steps between -70 and -90 mV, inward rectification was observed. The size of the inward current increased as the voltage step became more negative. Peak inward current amplitudes between 0.5 and 1.0 nA were observed and required several hundred msec to fully develop. Inspiratory premotor neurons in the dorsal respiratory group are known to receive synaptic inhibition during expiration. The degree to which these cells could be hyperpolarized, however, would be limited by inward rectification. This action would also affect the expression of other membrane currents such as A-current which requires membrane hyperpolarization to remove its inactivation. (Supported by NIH grant HL-39929)

471.11

EFFECT OF LESIONING THE PARABRACHIAL NUCLEI ON THE PHRENIC NERVE RESPONSE EVOKED BY NASAL MUCOSAL STIMULATION. T.E.Dick and N.S. Cherniack, Departments of Medicine and of Physiology and Biophysics, Case Western Reserve Univ., Cleveland, OH 44106.

Stimulation of the upper airways by noxious agents evoke changes in breathing pattern which presumably help defend the airways. The neural pathway for this reflex is unknown. However, the parabrachial nuclei receive a strong projection from paratrigeminal neurons and contain neurons that have phasic activity related to the breathing pattern. Collectively these neurons are referred to as the pontine respiratory group (PRG). We tested the hypothesis that the PRG is important in mediating respiratory defense reflexes by assessing the phrenic response to electrical stimulation of the nasal mucosa before and after electrolytic lesion of the parabrachial nuclei. Twenty anesthetized (chloralose-urethane, 10-50 mg/kg), paralyzed, and ventilated cats were studied. Stimulating electrodes were placed bilaterally in the nares and a iesioning microelectrode was placed in the rostral dorsolateral pons (A/P P 4, M/L 5, H -3 mm) at the site where phrenic nerve activity was inhibited by the smallest current (<50 uA, 1 ms) at a short latency (5-6 ms). Before lesioning the PRG, nasal mucosal stimulation (1 ms, 10 Hz) inhibited phrenic nerve activity transiently but completely. Threshold for phrenic inhibition by nasal stimulation varied between 0.2-1.0 mA with a mean latency of 8.9 ms. The PRG was lesioned bilaterally (10 mA, DC, 60 s). Effective lesions prolonged the duration of inspiration. In 12 animals, partial lesions of the PRG resulted in a decrease in the response and/or an increase in the threshold of the stimulus pulse necessary to inhibit the phrenic. We conclude that an intact PRG is necessary for the respiratory defense reflexes elicited by nasal mucosal stimulation. SUPPORT: USPHS-HL-25830.

471.12

HIGH PRESSURE REDUCES SENSITIVITY TO ALTERATION IN PH IN ISOLATED MEDULLA SPINAL-CORD OF NEWBORN RATS. A. Tarasiuk* and Y. Grossman. Unit of Physiology, Faculty of Health Sciences, Ben-Gurion University, Beer-Sheva 84105, Israel.

High pressure (HP) induces neurological symptoms associated with various respiratory difficulties. We examined the effect of HP on the sensitivity of the respiratory center to alteration in pH. The medulla and cervical spinal cord were isolated from anesthetized newborn rats, placed in a pressure chamber and superfused with 95% O_2 - CO_2 Ringer's solution. Solutions with pH of 6.8-7.6 were obtained by either equilibration with varied P_{CO_2}, or adjustment of the $[HCO_3^-]$. Respiratory related activity was recorded from C_1 and C_5 cut ventral roots. At atmospheric pressure, lowered pH increased the frequency of respiratory activity in both C_1 and C_5, more so for P_{CO_2} than $[HCO_3^-]$. Exposure to pH 6.8 (P_{CO_2}) reduced by 22% the time integral of a single respiratory burst in C_5, but not in C_1. HP reduced by 60% the sensitivity of the system to alteration in pH by both methods. In addition, the time integral of both C_1 and C_5 responses became independent of P_{CO_2} causing a relative change in "Respiratory Drive" (time integral x frequency) between the two responses. These modifications in the chemoresponse of the respiratory center may contribute to the respiratory problems encountered under HP conditions.

471.13

RESPIRATORY-LIKE ACTIVITIES IN THE ISOLATED VASCULARLY PERFUSED BRAINSTEM OF THE ADULT GUINEA PIG. M.P. Morin-Surun*, H. Sarraseca* and M. Denavit-Saubié, L.P.N.T - C.N.R.S., 91198 Gif-sur-Yvette, France.

In order to study the generation of respiratory activities in the brainstem we used the isolated perfused brainstem of adult guinea pig. The brainstem was rapidly removed from the cranium, the basilar artery was cannulated at the pontine level and was perfused rostro-caudally with O_2-CO_2 saturated Ringer's. The central respiratory drive was recorded from the ventral roots of the hypoglossal nerve since the rhythmic activities of hypoglossal and phrenic nerves are related "in vivo". Neuronal activities were recorded in the brainstem through a 2-barrelled electrode containing a dye to mark the recording sites. Different types of spontaneous rhythmic discharges were identified: 1) repetitive single spikes or bursting activity with a firing rate of 3-10/sec. 2) trains of 5-20 spikes separated by long and relatively constant intervals giving a spike-train frequency of 2-75 trains/min. Some of these latter periodic activities were respiratory-like since they were related to the periodic hypoglossal nerve activity. These rhythmic activities were recorded in the same brainstem nuclei where respiratory related units have been found "in vivo": dorsally in the nucleus tractus solitarius and ventrally in the ambiguus and paragigantocellular reticular nuclei. These results show that rhythmic function is present in the isolated brainstem which may be an useful tool to study the network responsible for respiratory generation.

471.14

SINGLE vs. MULTIPLE RESPIRATORY PATTERNS FROM THE ISOLATED BRAINSTEM / SPINAL CORD OF NEONATAL RATS. M. Takenoshita* and D. Russell. Dept. of Anesthesiology, Washington Univ., School of Med., St. Louis, MO 63110

The in vitro brain stem - spinal cord preparation of the neonatal rat is used to study mechanisms of respiratory pattern generation. The rhythms reported to date have consisted of slow single bursts (0.5-1 s duration, 0.1-0.2 Hz). We report here a different pattern, to be termed "multiple bursting", in which 3-25 consecutive bursts (0.3-1 s duration, 0.5-1 Hz) form a "group", and such groups appear periodically at 15-40 s intervals.

0-3 d rats were anesthetized, the head and spine were excised, and the pons, medulla, and spinal cord were isolated. Rhythmic respiratory discharges were recorded from a phrenic nerve and a C_1 ventral root using suction electrodes.

Multiple-bursting patterns were recorded in more than 80% of all preparations. Multiple-bursting tended to change to the single-burst pattern under the following conditions: 1) preparation aging, 2) hypoxia, 3) 2-3 mM Mg, 4) 100 µM GABA or 150 µM glycine, or 5) high temperature (37°C). In some cases, 0.5 mM Naloxone, 100 µM theophylline or 100 nM TRH could change single bursting to the multiple-burst pattern. Within a group, the initial burst had a different waveform than subsequent bursts, and resembled the discharges during the single-burst pattern with an abrupt onset at maximal amplitude.

The single-burst pattern appears to be too slow, and has an inappropriate waveform, to resemble normal breathing of intact neonates (diaphragm EMG: ~100 ms duration, 0.5-2 Hz); the single-burst pattern may instead represent gasping. The multiple-burst pattern is a better mimic of the normal in vivo breathing pattern.

471.15

RESPIRATORY AND CARDIOVASCULAR RELATED BRAINSTEM NEURAL ASSEMBLIES: DYNAMIC FUNCTIONAL CONNECTIVITY B.G. Lindsey, Y. M. Hernandez*, R. Shannon, and G. L. Gerstein, Dept. Physiol. & Biophys., Univ. South Florida, Tampa, FL 33612 and Dept. Physiol., Univ. Penn., Philadelphia, PA 19104.

Neurons with firing rates modulated in phase with the respiratory and/or cardiac cycles are distributed in the midline of the brainstem. The cooperative behavior of these neurons was studied in 18 anesthetized (Dial), paralyzed, bilaterally vagotomized, artificially ventilated cats. 73 samples of 4-9 simultaneously monitored midline neurons located in the regions of n. raphe obscurus and the pontine-medullary border were recorded and analyzed. Statistical significance of groups identified by gravitational clustering (Brain Res. 483:373) was determined by comparing particle aggregation in unshifted spike data with that derived from temporally shifted data sets. (1) Detected assemblies included elements synchronized with respiration, the cardiac cycle, or both rhythms. (2) Neurons of some assemblies were recorded in both rostral and caudal regions and were intermingled with elements of other assemblies. (3) Evidence was obtained for reciprocal connections between assemblies appropriate for generating observed respiratory phase dependent modulation of synchronous activity. The results suggest complex interdependencies in these networks. Supported by NS19814.

471.16

CARDIO-RESPIRATORY EFFECTS OF TETRODOTOXIN (TTX) IN URETHANE-ANESTHETIZED GUINEA PIGS. F.-C.T. Chang, B. Benton, J.S. Salyer and D.R. Franz. Pathophysiol. Div. US Army Med. Res. Inst. of Chem. Def. APG, MD 21010-5425

Cardio-respiratory effects of TTX (15 ug/Kg; single ip dose) were studied in urethane-anesthetized guinea pigs instrumented for concurrent monitoring of medullary respiratory-related units (RRUs), diaphragm EMG (DEMG), ECoG, EKG, blood pressure (BP), arterial O_2 and CO_2, end-tidal CO_2, vagal traffic and core temperature. TTX consistently produced a state of progressive hypercapnia which lasted the entire course of intoxication. System responses initially showed a hyperpneic profile. This was followed by a) a profound, time-dependent decrease in respiratory cycle frequency; b) an elevating RRU spike frequency; and c) a marked increase in expiratory RRUs' Te/Ti ratios and a modest decrease in inspiratory Ti/Te ratios which is indicative of fundamental changes in the bulbar respiratory rhythmogenic mechanism. Other changes included a slight but steadfast decline in a) BP; b) DEMG amplitude; and c) vagal traffic. EKG and core temperature remained unchanged up to the point of respiratory arrest. Simultaneously recorded inspiratory and expiratory RRUs showed that respiration invariably failed in an end-expiratory position. In conclusion, among the variables measured, the central rhythmogenic mechanism appears to exhibit the highest sensitivity to perturbation by TTX.

471.17

TOLERANCE TO ANOXIA IN NEWBORN MAMMALS AND REPTILES: INTRACELLULAR NEURONAL STUDIES. G.G. Haddad and D.F. Donnelly*. Dept. of Pediatrics., Yale Univ., New Haven, CT 06510.

Experiments on adult (A) and newborn (NB) rat and adult turtle brainstem slices were undertaken to determine the sensitivity or tolerance to hypoxia (PO_2= 10-15 Torr) of brainstem neurons. Intracellular recordings showed that A rat hypoglossal neurons (n=23) depolarize by 31.7 ± 9.2 mv during exposure to 5 min. of hypoxia while NB (age= 3-16d) (n=47) depolarize only by about 11.4 ± 5.6 mv. NB cells could be exposed to hypoxia for more than 15 min. before they depolarize by >20mv. The hypoxic depolarization in A is most likely a postsynaptic phenomenon since TTX, Ca++ free or low Ca++, high magnesium solutions did not affect the depolarization trajectory. [K+] in the brainstem extracellular compartment (ECF) increased much more in A (~3.0mM) than in the NB (~0.65mM) during hypoxia. Adult turtle brainstem neurons (n=7) tolerated complete anoxia for more than 30 min. at 23°C with little change (±3 mv) in membrane potential or firing frequency. Tolerance to anoxia in turtle neurons did not change when recordings were made at the same temperature (36°C) as that for rat recordings. We conclude that 1) the difference in the magnitude of depolarization during hypoxia between A and NB rat neurons is, at least in part, due to the difference in ECF [K+]; 2) the tolerance to O_2 deprivation in turtles is much greater than that of A or NB rats and 3) the increased tolerance in turtles is not related to the lower brain temperature in these animals.

471.18

ALTERATION OF RESPIRATORY CYCLE TIMING BY SINGLE PULSE STIMULATION OF THE HIPPOCAMPUS IN THE CAT. C.A. Richard, R.R. Terreberry and R.M. Harper. Brain Research Institute and Dept. of Anatomy & Cell Biology, UCLA, Los Angeles, CA 90024.

The hippocampal formation of the cat exhibits rhythmical slow electrical activity during periods of somatic activity, with the frequency and amplitude of this activity being a function of particular motor acts. Envelopes of this rhythmical activity are correlated with phasic diaphragmatic EMG bursts, with the phase relationship dependent on period length of phasic bursts. This apparent relationship of hippocampal electrical activity to respiratory patterning may be an epiphenomenon reflecting simultaneous activation of motor structures, or may suggest a role, perhaps of activation, in organizing breathing patterns. We examined the effect of single (300-400 uA) electrical pulses delivered to the dorsal hippocampus during the awake state of drug-free intact, freely moving cats. Pulses delivered at rates slightly higher than base respiratory rhythms induced a switch from expiration to inspiration. These results confirm a previous study demonstrating inspiratory switching effects of stimulation delivered to the hippocampus of anesthetized cats, and suggest a more active role for this structure in organizing respiratory patterns. Supported by HL22418-12.

471.19

SLOW WAVE ELECTRICAL ACTIVITY IN THE HIPPOCAMPUS: RELATION TO RESPIRATORY PATTERNING. R.M. Harper, C.A. Richard, R.C. Frysinger, R.R. Terreberry, A. Garfinkel* and R.K. Harper*, Brain Research Institute and Departments of Anatomy & Cell Biology and Kinesiology, UCLA School of Medicine, Los Angeles, CA 90024.

We previously described an amplitude modulation of hippocampal 4-8 Hz rhythmic slow activity correlated with respiratory patterning during waking and rapid eye movement sleep. This study examined slow wave electrical activity from the hippocampus during two conditions that modify respiratory patterning: quiet sleep, which extends inspiratory duration, and hyperthermia, which induces tachypnea. Five cats were instrumented with bipolar electrodes placed in the cortex and hippocampus; EMG and ECG leads were placed in the diaphragm and nuchal musculature. Following recovery, each cat was allowed to sleep undisturbed in a quiet chamber and later was administered cocaine to induce hyperthermia. Temperature was monitored with a rectal probe or with implanted brain or neck muscle probes. Electrical activity from the hippocampus was cross-correlated with integrated diaphragmatic activity. Hyperthermic episodes (core temperature about 39°C) were associated with very rapid respiratory rates and with enhanced involvement of upper airway muscles, including the genioglossus and facial musculature. In both quiet sleep and hyperthermic conditions, diaphragmatic activity was correlated with aspects of slow wave hippocampal electrical activity; both inspiratory and expiratory components of the respiratory cycle were reflected in hippocampal electrical activity during quiet sleep. We conclude that respiratory patterning is reflected in electrical activity of rostral brain areas and that different aspects of hippocampal electrical activity are related to alterations in respiratory pattern. Supported by HL22418.

ACETYLCHOLINE IV

472.1

QUANTITATIVE AUTORADIOGRAPHIC MEASURMENT OF NICOTINIC RECEPTOR BINDING SITES IN RAT BRAIN WITH ^{3}H-METHYLCARBAMYLCHOLINE. J.Y.Chang, W.K.Smith and D.J.Woodward, *Dept.of Cell Biol. UT Southwestern Med. Center and Biographics, Inc. Dallas,TX 75235.*

Brain nicotine receptors have been mapped autoradiographically by using different radioligands. Biochemical analysis of nicotine receptor binding properties has also been reported but not usually in individual brain nuclei. The present study was performed using a high resolution regional receptor analysis system (Biographics Inc.) to reveal quantitively the nicotinic receptor binding site properties in individual brain areas of rat. Brain sections were incubated with 1-20nM ^{3}H-methylcarbamylcholine for 60 min at 4°C in the presence of 3μM atropine. Unspecific binding was determined by adding 10μM cold nicotine in the incubation medium. Brain paste mixed with different concentration of ^{3}H-ornithine served as standards. Preliminary K_D and B_{max} values calculated by Scatchard plot analysis obtained from digitized autoradiographic data were (K_D=nM, B_{max}=fmol/mg protein):

	CPU	COR	AV	AM	LG	SUG	SNC	MG
K_D	3.24	3.64	8.11	10.88	3.33	8.13	2.85	5.51
B_{max}	67.7	79.10	266.9	237.9	110.0	188.2	82.0	130.2

(CPU=caudate putamen, COR=frontal cortex, AV=anterioventral thal nu, AM=anteriomedial thal nu, LG= lat geni nu, SUG=superfcial gray layer of sup col, SNC=substantia nigra,pars compacta, MG=med geni nu). K_D values are consistent with, but B_{max} values are higher, than data obtained from biochemical measurments (Lapchak, P. A., J.Neurochem. *52*:483,1989). Chronic injection of nicotine for 40 days (0.4mg/kg twice daily, s.c.) tended to increase nicotine binding in some brain areas. The results provide detailed anatomic information about nicotine receptor binding in CNS and will be useful in the continued study of dynamic changes in the properties of nicotine receptor binding that are induced by different pharmacological treatments. *(Supported by DA2338, NIAAA3901, R.J.R.Tobacco Co., NINDS NS-25321 and Biol. Humanics Found.)*

472.2

3H-MECAMYLAMINE BINDING AND THE BRAIN NICOTINIC RECEPTOR. S. Banerjee*, J.S. Punzi*, L.G. Abood, Dept. Pharmacology Univ. of Rochester, Rochester, NY 14642.

Mecamylamine (Mc), antagonizes the action of nicotine and other nicotinic agonists both at autonomic ganglia and in brain; but it does not appear to act either competitively or allosterically at nicotinic receptors, using ^{3}H-nicotine as the ligand. With the use of ^{3}H-(N-methyl)-mecamylamine a study was undertaken to characterize the site of Mc's action using synaptosomes. Specific ^{3}H-Mc binding was maximal in 10-20 sec and declined to equilibrium in 1 min. A Scatchard yielded a K_d value of 2 nM and B_{max} of 29 fmoles/mg protein; and the K_d determined from the association and dissociation rate constants was 0.4 nM. A good correlation was noted between the K_i values of a number of Mc analogues and their ability to block the peripheral and central actions of nicotine. The apparent K_i values were 0.8 nM for the 3-dimethyl, 4 nM for the desmethyl, 5 nM for the 3-benzyl analogues of Mc. Although Mc did not compete with ^{3}H-nicotine binding to synaptosomes or membranes, nicotine and a number of its analogues competed with ^{3}H-Mc for binding with K_i values 20 nM but their affinities did not correlate with the psychotropic potency of the analogues. The findings are consistent with the hypothesis that 1) Mc exerts nicotinic blockade at distinct (ionic channel) sites, and 2) nicotine itself may be exerting its pharmacologic effects by acting both at nicotinic receptors and the ionic sites.

472.3

PILOCARPINE: A CONFORMATIONALLY FLEXIBLE MUSCARINIC AGONIST WITH M_1 AND M_2 ACTIVITIES IN THE CNS. W. Hoss, J.M Woodruff*, B.R. Ellerbrock*, S. Periyasamy*, S. Ghodsi-Hovsepian J. Stibbe* and W.S. Messer, Jr. Department of Medicinal and Biological Chemistry, College of Pharmacy, University of Toledo, Toledo, OH 43606

Pilocarpine is well-established as a muscarinic agonist, displaying both M_1 (gut) and M_2 (heart) effects in the periphery. Central M_2 effects include lowering of body temperature and tremors at high concentrations. Central M_1 effects of pilocarpine are less well established. Binding studies using [^{3}H]-QNB in coronal sections of rat brain revealed that pilocarpine was one of the least M_2-selective muscarinic agonists, having the following rank order of subtype selectivity: M_2(superior colliculus)>M_3(rhomboid thalamus)>M_1(dentate gyrus)>M_4(substantia nigra), with the ratio $M_2/M_1 = 6$. Pilocarpine was tested biochemically *in vitro* for its ability to stimulate PI turnover in the hippocampus (M_1 response) where it displayed 35% of the maximal carbachol response with an EC_{50} value of 18 µM, and low-K_m GTPase in the cortex (M_2 response), where it had 73% of the maximal carbachol response. Behaviorally, pilocarpine was able to restore deficits in a representational memory task (sensitive to M_1 antagonists) produced by intrahippocampal injections of AF-64A. Twenty-three low-energy (within 20 kj of the global minimum) conformations of protonated pilocarpine were generated using the MM2 forcefield implemented on C. Still's program Macromodel. One conformation (second lowest energy) corresponded closely (rms=0.189 A) to the structure of pilocarpine hydrochloride obtained by X-ray crystallography. Structure refinments and charge densities were calculated using M.J. Dewar's MNDO method implemented on the program MOPAC. The positive charge was diffusely spread over various C and H atoms of the imidazole ring, whereas both N and both O atoms bear partial negative charges. It is conceivable that different conformations of pilocarpine are active as agonists at different muscarinic receptor subtypes. Supported by NS 23929 and NS 25765.

472.4

POTENTIATION OF CHOLINERGIC ACTIVITY WITH PYRIDINO [1,2-a] IMIDAZO [5,4-b] INDOLE. H.K. Rucker, E.L. Moore*, P.K. Adhikary* and M.A. Maleque*. Neuroscience Group, Meharry Medical College, Nashville, TN 37208.

The effect on cholinergic function of a novel imidazoindole derivative, pyridino [1,2-a] Imidazo [5,4-b] Indole (IMID), was assayed with *in-vitro* preparations of guinea pig ileum, frog sciatic nerve-sartorius muscle, and denervated rat soleus muscle. The effect on cholinesterase activity was evaluated in rat blood and homogenized ileal tissue. Additionally, the compound was applied *in-vivo* by iontophoresis to cholinoreceptive neurons in rat auditory cortex responding to acoustical stimulation. The imidazoindole derivative was prepared in the laboratory according to the procedure previously described (J. Med. Chem 19:1352-1354, 1976).

IMID alone at 10^{-11} to 10^{-5}M concentrations did not increase developed tensions in ileal tissue; however, potentiation of 10^{-8}M acetylcholine (ACh) evoked developed tension ranged from +20% to +60%. Responses were blocked by 10^{-8}M atropine. Indirect (nerve) stimulated sartorius muscle twitches demonstrated a half-maximum effect at 115% of control with 5 x 10^{-5}M IMID. No effect was noted on twitches evoked by direct stimulation. In denervated rat soleus muscle, up to a 38% potentiation in tension was demonstrated with IMID plus ACh versus ACh alone. In the rat cortex, the responses to characteristic frequency tones were augmented (40-122%) with IMID plus ACh versus ACh alone. The compound had no effect on cholinesterase activity.

The results suggest a potentiation of cholinergic function mediated through an effect on the post-synaptic ACh-receptor complex; however, an effect on acetylcholine release cannot be ruled out. [Supported in part by: NIH-S06RR08037-17; NSF-BNS8617837; and VAMC RAG program.]

472.5

NEUROPEPTIDE EFFECTS ON MUSCARINIC RECEPTORS IN RAT FRONTAL CORTEX. M. A. Rice*, J. P. Carney*, L. D. Oswari*, and N. W. Pedigo. Depts. Pharmacology and Anesthesiology, Univ. Kentucky. Med. Ctr., Lexington, KY 40536.

The objective of this research was to evaluate potential regulation of muscarinic receptors by selected neuropeptides. Neuropeptides were chosen from those which co-exist in forebrain cholinergic neurons or are altered in age-related disorders such as Alzheimer's disease, including vasoactive intestinal polypeptide (VIP), cholecystokinin (CCK), somatostatin (ST), and neurotensin (NT). Muscarinic receptors were characterized by radioligand binding techniques using ^{3}H-quinuclidinyl benzilate (^{3}H-QNB) measured in rat frontal cortex. Cortical membranes were co-incubated with ligand (^{3}H-QNB, 5-1500 pM), a single neuropeptide (0.1 uM) in the presence or absence of atropine (10 uM) and incubated to equilibrium for 1 hour at 37°C. Saturation studies in control preparations yielded binding consistent with a single population of sites having Kd = 61 ± 6 pM and Bmax = 97.7 ± 5.1 fmol/mg tissue (mean ± SEM, n = 14). The neuropeptides VIP, CCK, ST and NT did not significantly affect Kd (89, 71, 99 and 135% of control, respectively) or Bmax (95, 91, 126 and 104% of control). Further studies in tissue from senescent rats will define possible age-related modulation of muscarinic receptors by neuropeptides after in vitro and in vivo exposure. (Supported by the Frank H. Gower Memorial Fund, Alzheimer's Disease and Related Disorders Assoc. grant)

472.6

AUTORADIOGRAPHIC STUDIES OF AGONIST-BINDING TO M1- AND M2 MUSCARINE RECEPTORS E.Ø. Nielsen*, V. Petersen* and T. Honoré* (SPON: M. TREIMAN) Ferrosan CNS Division, DK-2860 Soeborg, Denmark.

Studies of the binding properties of M1 muscarine receptors have been more difficult than similar studies of M2 receptors, because of the lack of a M1 specific agonist. The different affinity of muscarine receptors for agonists and antagonists has made binding studies using the selective M_1 antagonist ^{3}H-pirenzepine very difficult to interpret. ^{3}H-Oxotremorine-M (^{3}H-oxo) is believed to label both M1- and M2 receptors but so far it has not been possible to show regional distributions in autoradiographic studies in accordance with this hypothesis. Very low binding is observed in areas rich in M1 receptors i.e. hippocampus whereas high levels are found in superior colliculus (sc) which is rich in M2 sites. By using a very short washtime we have improved ^{3}H-oxo binding to M1 receptors. Apparently the on and off rates are much faster for M1- than M2 receptors. Saturation experiments showed binding to a single class (mean n_H=0.98) of high affinity (mean K_D=5.9 nM) sites. Nevertheless, inhibition of ^{3}H-oxo binding by pirenzepine give biphasic displacement curves showing area dependent percentages of M1- and M2 sites. Dentate gyrus is rich in M1 sites (K_H=6 nM (60%), K_L=9 µM (40%)) whereas sc is pure M2 sites (K_L=11 µM (100%)). The affinties for central agonist M1- and M2 receptors of selected standard compounds have been measured by blockade of ^{3}H-oxo binding to rat brain sections in dentate gyrus and sc.

472.7

INVOLVEMENT OF Na^+/H^+ EXCHANGE SYSTEM, PHOSPHOLIPASE A-2 AND LIPOXYGENASE PATHWAY IN THE MODULATION OF RAT STRIATAL [^{14}C]-CHOLINE UPTAKE AND [^{3}H]-HEMICHOLINIUM-3 BINDING. T.K. Chatterjee* and R.K. Bhatnagar. Dept. Pharmacology, University of Iowa, Iowa City, IA 52242

Sodium-dependent choline uptake regulates the synthesis of acetylcholine. The processes which impart sodium dependency of choline uptake remain unclear. Since the Na^+/H^+ exchange system and phospholipase A-2 activity are linked in many cellular events, their role was investigated in [^{14}C]-choline uptake and [^{3}H]-hemicholinium-3 (HC-3) binding in rat striatal synaptosomal and membrane preparations, respectively (Chatterjee et al., Europ. J. Pharmacol. 149:241, 1988; J. Neurochem. 49:1191, 1987). Amiloride, an inhibitor of Na^+/H^+ exchange system, inhibited both [^{14}C]-choline uptake and [^{3}H]-HC-3 binding with IC_{50} (µM) of 444 and 332, respectively. Quinacrine, an inhibitor of phospholipase A-2, inhibited choline uptake with an IC_{50} of 2 µM but had no effect on [^{3}H]-HC-3 binding. Indomethacin (100 µM), the inhibitor of cycloxygenase pathway of arachidonic acid metabolism, did not affect either the [^{14}C]-choline uptake or [^{3}H]-HC-3 binding. Inhibitors of lipoxygenase pathway, nordihydroguaiaretic acid, guanabenz and ascorbic acid 6-palmitate inhibited choline uptake with IC_{50} (µM) of 34, 16 and 49, respectively. We suggest that the Na^+/H^+ exchange system, phospholipase A-2 activation and generation of arachidonic acid metabolites through the lipoxygenase pathway are involved in the regulation of synaptosomal choline uptake. Supported by DAMD Contract # 17-87-C-7113.

472.8

EFFECTS OF BETA-CARBOLINES ON HIGH AFFINITY CHOLINE UPTAKE IN CORTICAL AND HIPPOCAMPAL SYNAPTOSOMES. J.A. MILLER and P.A. Chmielewski, Merrell Dow Research Institute, Cincinnati, OH, 45215.

GABA-antagonists play a role in stimulating basal forebrain cholinergic neurons. A recent account (Nakahiro, M., et al., Br. J. Pharmacol. 95, 1303, 1988) indicates that a nootropic agent, pantoyl-GABA, stimulates high affinity choline uptake (HACU) and that this effect may be responsible for its ability to enhance cognition. Several benzodiazepine inverse agonists were administered to rats i.p., the hippocampi and cerebral cortices were removed and HACU was measured in a synaptosomal preparation.

A stimulation of HACU was seen in the cortex with a convulsant dose of picrotoxin and convulsant and subconvulsant doses of several beta-carbolines. In the hippocampus, however, while picrotoxin and methyl 6,7-dimethoxy-4-ethyl-beta-carboline-3-carboxylate (DMCM) stimulated HACU, ethyl beta-carboline-3-carboxylate (beta-CCE) and methyl beta-carboline-3-carboxylate (beta-CCM) did not. Additionally, the partial inverse agonist, N-methyl-beta-carboline-3-carboxamide (FG-7142) stimulated HACU in the cortex after in vivo administration. HACU in the hippocampus was unaffected by FG-7142. Another related compound, 3-hydroxymethyl-betacarboline (3-HMC) had no effect on HACU in either the cortex or hippocampus at a dose of 20 mg/kg. This suggests some selectivity between these two populations of cholinergic neurons.

472.9

PARTIAL PURIFICATION OF A [^{3}H]HEMICHOLINIUM-3 BINDING SITE FROM BOVINE STRIATUM. R.L. Shocket, K. Yamada, M.D. Saltarelli, V. Balasubramanian*, H. Wagner*, A. Soliman*, and J.T. Coyle. Dept. of Neuroscience, The Johns Hopkins University School of Medicine, Baltimore, MD 21205.

[^{3}H]hemicholinium-3 ([^{3}H]HCh-3), a potent inhibitor of sodium-dependent high affinity choline uptake (SDHACU), the rate-limiting step in acetylcholine synthesis, binds to the carrier of SDHACU with high specificity. In the present study, we examined the [^{3}H]HCh-3 binding site in bovine striatal membranes. The specific binding of [^{3}H]HCh-3 in bovine membranes exhibited sodium-dependency and was inhibited by HCh-3 and choline with an IC50 of 5.9 nM and 69 µM, respectively. The [^{3}H]HCh-3 binding site was solubilized by 0.2 % deoxycholate in the presence of 200 mM NaCl. The addition of ten percent glycerol improved stability, and the binding activity remained constant up to one month when kept at -80°C. The solubilized carrier was applied to a DEAE-Sepharose column after the detergent was exchanged to 0.001% Tween 80. Protein was eluted by a linear gradient of NaCl, and the peak of binding activity was found in the fractions containing approximately 400 mM NaCl. The solubilized carrier was also applied to a sepharose-4B adipic HCh-3 affinity resin through batch incubation in the presence of 200 mM NaCl. The resin was column washed extensively and binding activity was eluted with a no sodium, 1 mM choline containing buffer. On the basis of the amount of protein loaded on the affinity column and the amount recovered in the eluant associated with [^{3}H]HCh-3 binding, this step achieved a several thousand-fold purification.

472.10

COMPARATIVE STUDY OF PASSIVE AND ACTIVE ACETYLCHOLINE UPTAKE BY SYNAPTIC VESICLES. K. Norenberg and S.M. Parsons. Department of Chemistry, University of California, Santa Barbara CA 93106.

A comparative study was performed on passive and active acetylcholine uptake of synaptic vesicles isolated from the electric organ of Torpedo californica. Active transport mediated by the acetylcholine transporter requires ATP, is only slightly inhibited by prior formation of vesicles ghosts, and is strongly inhibited by vesamicol, cold, low pH and 4-chloromercuriphenylsulfonate(MPS). Passive uptake is slightly inhibited by ATP and cold, nearly unaffected by vesamicol and is stimulated by low pH, MPS and other sulfhydryl modifiers and prior formation of vesicles ghosts. The ion dependence of passive and active acetylcholine transport is also diffrent. It is concluded that passive uptake of acetylcholine is not mediated by the acetylcholine transporter but rather by a channel.

472.11

AMINOPYRIDINES INCREASE ACETYLCHOLINE RELEASE FROM STIMULATED BRAIN SLICES WITHOUT ACCELERATING PHOSPHOLIPID DEPLETION. R. L. Buyukuysal*, T. C. Holmes*, R. J. Wurtman (Spon: H. R. Lieberman). Department of Brain and Cognitive Sciences, Massachusetts Institute of Technology, Cambridge, MA 02139

We examined the effects of aminopyridines [4-aminopyridine (4-AP) and 3,4-diaminopyridine (3,4-DAP)] on acetylcholine (ACh) release from rat striatal slices superfused with a choline (Ch) containing or Ch free medium, at rest and during electrical stimulation. In Ch free medium, 4-AP (10-100 uM) or 3,4-DAP (1-10 uM) increased basal ACh release, while lowering the net efflux of Ch; thus, while the sum of ACh and Ch released remained constant, the ratio of ACh to Ch release was increased. Tissue ACh, Ch, and membrane phospholipid levels [including phosphatidylcholine (PC)] were not affected by aminopyridines. In a Ch (40uM) containing medium, aminopyridines further potentiated Ch-induced ACh release. Electrical stimulation of striatal slices increased ACh release without altering Ch efflux and depleting tissue Ch-ACh stores, but depleted membranes of PC and other major phospholipids. Superfusion of the striatal slices with aminopyridines during stimulation enhanced ACh release; diminished Ch efflux; and protected the slices from stimulation-induced phospholipid depletion. Calcium dependent activation of high affinity choline uptake may underlie the observed effects of the aminopyridines. (Supported in part by grant MH-28783.)

472.12

NEURONAL MICROTUBULES OBSERVED BY THE SCANNING TUNNELING MICROSCOPE. S.R. Hameroff, Y. Simic-Krstic, M. Kelley, C. Schneiker, M. Krasovich, R. McCuskey. Departments of Anesthesiology, Anatomy and Optical Sciences, Univ. of Arizona, Tucson, Arizona 85724.

Scanning tunneling microscopy (STM) can image atomic surfaces of metals and semiconductors. Biological STM applications are somewhat limited by poor conductivity, adsorbate layers, elasticity and poor stability of biomolecules. For direct STM observation of microtubules (MT) isolated from pig brain by standard techniques of differential ultracentrifugation, we determined optimal preparation conditions: fixation with 0.1% glutaraldehyde and solution in 0.8 M glycerol reassembly buffer (Mes, EGTA, GTP, $MgCl_2$). Both freeze dried and hydrated MT prepared in this way were reproducibly imaged in air at room temperature on graphite with a Nanoscope I STM (Digital Instruments, 135 Nogal Drive, Santa Barbara, CA 93110). The presence of MT was verified by electron microscopy. STM probing showed structures 25 nm in width, consisting of 5 to 7 longitudinal filaments of about 4 nm width: top views of MT which show about half of their 13 component protofilaments. Although MT appeared semiflattened, the known helical twist of protofilaments was clearly evident. Top view shaded scans revealed 4x8 nm individual tubulin subunits within protofilaments. STM and related techniques(atomic force microscopy, scanning near field optical microscopy, scanning ion microscopy) offer unique opportunities for the study of neuromolecular structures.

472.13

COMBINATION OF SHORT INTERVAL BRAIN DIALYSATE COLLECTION AND WGA-HRP ADMINISTRATION IN RAT. L. Gorman, B. Shook, and D. Becker. BRI, Depts of Neurosurgery and Anatomy & Cell Biology, UCLA Med. School, Los Angeles, CA 90024.

Modifications of an existing HPLC acetylcholine (ACH) assay were made to provide the time resolution needed to detect changes in the kinetics of ACH release during behavior. WGA-HRP was then administered through the same microdialysis probe that had been previously used for collection, allowing the anatomical localization of ACH neurons of origin.

Dialysate samples were collected at 5 minute intervals by altering the mobile phase and loading procedures of the analytical column and enzyme reactor. The length of the pre-injector guard column was changed from 50 mm to 10 mm. These changes permitted the femtomole range sensitivity levels, as reported by Damsma (1987), using commercially available equipment.

Subsequent to dialysis, the probe was raised and saturated with a 2% WGA-HRP (in pH 8.0 Tris buffer). The enzyme was then injected with a CMA/100 microinjection pump (50-100 nl at a rate of 1-2 nl/min). Brains were frozen sectioned and reacted for HRP (TMB histochemistry). HRP filled neurons were localized in various areas of the basal forebrain known to contain ACH cells.

472.14

EFFECTS OF AGING ON CHOLINERGIC AUTORECEPTOR FUNCTION IN THE RAT BRAIN. D. M. Araujo, P. A. Lapchak, M.J. Meaney, B. Collier, R. Quirion. Douglas Hospital Research Ctr. & McGill Univ., Montreal, Quebec, Canada H4H 1R3.

In the present study, we attempted to clarify whether cholinergic receptor density is altered in normal aging and whether this is reflected in a change in function. In homogenates of brain tissue from rats aged 3-, 9-, and 27-month old, we found that the total density (Bmax) of muscarinic sites, assessed using ^{3}H-QNB as ligand, is not altered in any brain region tested. However, the Bmax for ^{3}H-AF-DX 116 (muscarinic-M2) binding was significantly reduced in the 9-month old rats(29-55%), and further decreased in the 27-month old group. The density of muscarinic-M1 receptor sites was only modestly decreased in the old rats. In contrast, there was a marked reduction in the density of ^{3}H-MCC/nicotinic sites in the cortex, hippocampus, and striatum, but not cerebellum of both 9- and 27-month old rats. In slices of cortex and hippocampus, exogenous MCC significantly increased (by 42-73%) the spontaneous release of endogenous ACh, an effect which was not altered with age. In these same brain areas, AF-DX 116, a muscarinic antagonist, failed to enhance the K^+-evoked release of ACh in the 27-month old rats. In striatal slices,however, the AF-DX 116-induced augmentation of ACh release was similar in the young compared to the old animals. In conclusion, although both nicotinic and muscarinic-M2 sites are decreased with age, only muscarinic autoreceptor function is compromised.

472.15

MODULATION OF CORTICAL ACETYLCHOLINE RELEASE BY CHOLINERGIC AGENTS: AN IN VIVO DIALYSIS STUDY. J. Richard, D.M. Araujo and R. Quirion, Douglas Hospital Research Centre 6875 LaSalle Blvd Verdun, Quebec, Canada H4H 1R3.

Recent evidence has suggested the presence of positive (nicotinic) and negative (muscarinic M_2) autoreceptors controlling the synthesis and release of acetycholine (Ach) on cholinergic neurons in cortex and hippocampus. However, most of these experiments have been performed using in vitro preparations. The aim of the present study was thus to investigate if similar regulatory mechanisms can be demonstrated in vivo. Anesthetized male Sprague-Dawley rats (230-250g) were stereotaxically implanted with a transcortical dialysis probe (cut off 10,000); one to two days later, the release of endogenous Ach (in presence of 75uM physostigmine) was evaluated in non-anesthetized freely moving animals. Ach levels were determined using a gas chromatography/mass spectrometric assay (PCI). Nicotine (1-5mg/kg, sc) and atropine (1.0uM) were able to stimulate Ach release although not as potently as high K^+ (100mM) (2-3) fold over baseline). More interestingly, the combinaison of nicotine (to stimulate the positive autoreceptor) and atropine (to block the negative autoreceptor) was extremely potent in inducing the release of Ach (up to 10 times over baseline). This clearly shows that autoreceptors are present and active in vivo on cortical cholinergic neurons. It also demonstrates the tremendous capacity of these neurons in releasing Ach following appropriate stimulations (MRCC, Canada).

472.16

ACETYLCHOLINE CONTENT AND COMPARTMENTATION IN RAT CORTICAL SYNAPTOSOMES AFTER IN VIVO EXPOSURE OF BASAL FOREBRAIN CHOLINERGIC NEURONS TO QUINOLINIC ACID. R.H. Metcalf, D.L. Riddell* and R.J. Boegman. Dept. of Pharmacology and Toxicology, Queen's University, Kingston, Ontario, Canada K7L 3N6.

In this study, alterations in the concentration and subcellular distribution of cortical synaptosomal acetylcholine (ACh) after QUIN injection into the nucleus basalis magnocellularis (NbM) was investigated. Rats injected with either 600 or 1000 nmoles of QUIN were assayed for their synaptosomal ACh content at 0.5 h or 3.0 h post-injection. ACh concentrations in the P_2, P_3 or S_3 fractions were determined by gas chromatography-mass spectrometry. ACh concentration in the P_2 fraction of normal animals was 235±18 pmol/mg protein. Of this, 64 ± 16% was recovered in the P_3 fraction and 24±1% was recovered in the S_3 fraction. In comparison to naive rats, cortical synaptosomes (P_2) prepared 0.5 h post-injection contained significantly higher concentrations of ACh for both QUIN doses. At 3h post-injected synaptosomal ACh decreased sharply in rats treated with 1000 nmoles QUIN. As well, a shift in the subcellular distribution of ACh to the cytoplasmic fraction was seen with a 1000 nmole dose of QUIN. These results show that QUIN stimulation of nBM cortical projection neurons, produce both dose-dependent and time-dependent changes in synaptosomal ACh concentrations and subcellular distribution. Supported by the Medical Research Council of Canada and Ontario Mental Health Foundation.

472.17

CHARACTERIZATION OF N-METHYL-D-ASPARATE-MEDIATED ACETYLCHOLINE RELEASE IN RAT MEDIAL SEPTAL AREA. L.M. Nishimura* and R.J. Boegman. Department of Pharmacology and Toxicology, Queen's University, Kingston, Ontario, Canada, K7L 3N6.

Release of acetylcholine (ACh) in the medial septal area (MSA) may originate from collaterals of cholinergic septohippocampal neurons. Receptors for N-methyl-D-asparate (NMDA) have been located on these septohippocampal neurons and NMDA has been shown to stimulate the release of ACh in several areas of the central nervous system, thus NMDA may have an effect on ACh release in the MSA. The presence of the peptide galanin in this area suggests an additional role for galanin in modulating septohippocampal activity.

The objective of this study was to characterize the NMDA-mediated release of ACh from slices of rat MSA. NMDA evoked the release of ^{3}H-ACh from superfused slices of the MSA in the dose-dependent manner, with an apparent EC50 value of about 100 µM. This NMDA-induced release was significantly reduced by both MK-801 (100 nM) and galanin (500 nM). Tetrodotoxin (500 nM) was demonstrated to have an inhibitory effect on the NMDA-induced ^{3}H-ACh release from septal slices as well. These findings indicate that the ACh release in rat MSA is mediated through NMDA receptor stimulation and is modulated by galanin. In addition, ACh release in rat MSA appears to originate from axon collaterals of the septohippocampal pathway. This work was supported by Medical Research of Canada.

472.18

DETERMINATION OF ACETYLCHOLINE RELEASE BY MICRODIALYSIS IN THE RAT STRIATUM WITHOUT THE USE OF CHOLINESTERASE INHIBITOR. Koichiro Kawashima, Toru Hayakawa*, Takeshi Suzuki*, Kazuko Fujimoto* and Hisayo Oohata*. Department of Pharmacology, Kyoritsu College Pharmacy, Tokyo 105, Japan.

Because of limit of sensitivity, conventional methods require the use of physostigmine (PHY) for determination of acetylcholine (ACh) release by microdialysis in the brain. Using a sensitive and specific radioimmunoassay, control mechanism of ACh release was studied in male Wistar rats instrumented with a microdialysis probe in the striatum under anesthesia with ether and α-chloralose. Drugs were dissolved in artificial cerebrospinal fluid and perfused at a rate of 2 ul/min. Basal ACh release in the absence and presence of PHY (10 uM) was 5.6 ± 0.6 and 49.3 ± 5.7 fmol/min (n = 8), respectively. ACh release was not affected by the addition of atropine (0.1-10 uM) in the absence of PHY while it was increased in the presence of PHY. In the presence of PHY, pirenzepine (0.01-1 uM) increased ACh release while AF-DX 116 (0.01-1 uM) had no effect on ACh release. These results indicate that autoinhibition of ACh release operates only under specific conditions where local ACh concentration is elevated extremely, and that presynaptic M_1 receptor is involved in the regulation of ACh release.

472.19

MODULATION OF CARBACHOL-STIMULATED PHOSPHOINOSITIDE (PI) METABOLISM BY SOMATOSTATIN AND PHORBOL ESTERS. L. M. Shaffer* and L. A. Dokas, Depts. of Biochemistry and Neurology, Medical College of Ohio, Toledo, OH 43699.

In rat hippocampal slices, carbachol (CCh, a cholinergic agonist) stimulates PI metabolism, forming inositol triphosphate (IP_3), and diacylglycerol, which activates protein kinase C (PKC). In the presence of [^{3}H]-inositol and Li^+, IP_3 breakdown is blocked at the conversion of inositol phosphate (IP) to inositol and phosphate so that accumulation of [^{3}H]-IP serves as a measure of PI breakdown. Somatostatin (SS) may modulate cholinergic actions on this system by altering phosphorylation of a protein (B-50), a possible regulatory factor in the PI cycle. CCh elicits a 3-fold stimulation of IP accumulation. Oxotremorine produces less stimulation than CCh, and both atropine and pirenzepine block CCh stimulation, indicating M1 receptor involvement. [D-Trp^8]-SS added to slices with protease inhibitors acts as a weak agonist. Additive effects of CCh and [D-Trp^8]-SS were seen only at submaximal doses of CCh, indicating the peptide may exert its effect through modulation of cholinergic mechanisms. The active phorbol ester, TPA (12-O-tetradecanoyl-phorbol-13-acetate), a stimulator of PKC, blocks the cholinergic-stimulated response, implying intermediate steps of protein phosphorylation. Simultaneously, TPA alters phosphorylation of two proteins of 87 kD and 48 kD (B-50), both known PKC substrates. Supported by NIH grant NS 23598.

472.20

EFFECTS OF ACETYL-l-CARNITINE ON ACETYLCHOLINE SYNTHESIS IN RAT BRAIN SYNAPTOSOMES AND IN PC12 CELLS. H.L. WHITE AND P. W. SCATES*. DIVISION OF PHARMACOLOGY, WELLCOME RESEARCH LABORATORIES, RESEARCH TRIANGLE PARK, NC 27709.

Acetyl-l-carnitine (AlCar) is reported to improve cognitive function related to cholinergic deficits in aged rats and in human senility. In the present study, effects of AlCar on acetylcholine (ACh) synthesis were determined in synaptosomal preparations and in PC12 cells. Synthesis of [^{14}C]ACh from [^{14}C]glucose was stimulated by AlCar in crude rat brain synaptosomes (20 ± 1% stimulation at 10 µM AlCar). No effect of AlCar on purified choline acetyltransferase (ChAT) was observed in the same concentration range. When PC12 cells were incubated for 3 days in the presence of 10 to 100 µM AlCar, levels of ChAT were increased approximately 3-fold. In PC12 cells incubated with low concentrations of nerve-growth factor, ChAT was additionally stimulated by AlCar.

These results are consistent with a role for acetyl-l-carnitine in facilitating transfer of acetyl groups across mitochondrial membranes, thus regulating the availability in the cytoplasm of acetyl-CoA, a substrate for acetylcholine synthesis. They also support a possible utility of acetyl-l-carnitine in the treatment of cognitive deficits.

472.21

RELEASE OF MEMBRANE-BOUND CHOLINE O-ACETYLTRANSFERASE (EC 2.3.1.6) FROM RAT HIPPOCAMPAL TISSUE BY PHOSPHOLIPASE C FROM BACILLUS CEREUS. P.T. Carroll and L.K. Smith*, Texas Tech University Health Sciences Center, Lubbock, TX 79430

Although the majority of the enzyme choline O-acetyltransferase (EC 2.3.1.6; ChAT) in central cholinergic neurons is believed to be soluble, some also appears to be non-ionically associated with membranes (Benishin & Carroll, J. Neurochem, 41: 1030, 1983). How ChAT is associated with membranes is unknown. Recent reports suggest that some membrane-bound enzymes are covalently associated with membranes through phosphatidylinositol, a linkage that is sensitive to cleavage by phospholipase C from certain bacteria (PI-PLC). In the present study, we tested the hypothesis that ChAT might be bound to membranes in this way by incubating rat hippocampal tissue with PI-PLC from B. cereus. These results indicated that PI-PLC selectively augmented the release of ChAT. When PI-PLC treated tissue was subjected to Triton X-114 phase separation, a procedure that separates amphiphilic from hydrophilic proteins, the detergent soluble, membrane-bound fraction of ChAT appeared to be the source of the ChAT released by PI-PLC. Zinc, an inhibitor of PI-PLC, not only blocked the PI-PLC induced release of ChAT, but also that release of ChAT which was temperature dependent. These results suggest that some hippocampal ChAT is associated with membranes through a PI-PLC sensitive linkage. (Supported in part by NINCDS-2R01 NS 21289-05)

CONTROL OF POSTURE AND MOVEMENT VIII

473.1

DIFFERENCES BETWEEN CEREBRAL PALSY AND CONTROL CHILDREN ON KINEMATIC VARIABLES IN A DRAWING TASK. Hanneke van Mier, Wouter Hulstijn & Paul Westzaan (SPON: M. Clare). Nijmegen Institute for Cognition Research and Information Technology (NICI), University of Nijmegen, Nijmegen, The Netherlands.

Many tests for the assessment of movement disorders contain a few writing or drawing tasks. Usually measuring the performance on these tasks can only be done by judging the quality of the lines drawn on paper. Recording of the pen movements by means of an XY-tablet (digitizer), provides more objective measurements of drawing quality -errors, curvature of the lines, etc.- and adds many kinematic movement parameters, like velocity, pen pressure, pauses, etc.

In order to assess the possible contribution of these kinematic variables a group of 15 children with cerebral palsy (CP) and a matched control group of 15 normal children were given a number of drawing tasks. These tasks consisted of drawing between two straight or curved parallel lines, making zigzag horizontal and vertical movements connecting small squares, and a Fitts' type of task in which small circles had to be connected to a target, differing in size, distance and direction. In all tasks speed of drawing was stressed.

Large differences between the two groups were found in nearly all kinematic variables. Particularly striking were the results on a "fluency"-measure, i.e. the number of changes (maxima) in velocity, in the duration of the pauses between the drawing of successive individual lines, and in the number of stops. Kinematic variables helped to differentiate between CP-children whose drawing results on paper looked very similar.

473.2

DURATION OF MOVEMENT-RELATED EMG ACTIVITY IN PATIENTS WITH MILD CEREBELLAR DYSFUNCTION. S.H.Brown, H.Hefter*, J.D.Cooke, H.-J.Freund . Neurologische Klinik, Univ. Dusseldorf, Dusseldorf, FRG

Recent studies have shown that cerebellar patients are unable to appropriately grade acceleration duration. Since acceleration duration has been shown to be related to the duration of the initial agonist burst (AG1), we have investigated this relation in 5 patients diagnosed with bilateral cerebellar degeneration.

Subjects performed 10 - 70 deg step tracking movements and 30 deg alternating movements at frequencies from 0.5 Hz to the maximum possible for each subject.

Cerebellar patients graded AG1 and acceleration durations in step tracking movements up to acceleration durations of about 300 ms. Unlike normal subjects, they did not increase either AG1 or acceleration duration beyond this limit. In contrast, patients could increase both AG1 and acceleration duration beyond 300 ms during performance of alternating movements.

Cerebellar patients are thus capable of producing long duration muscle activation and resulting long duration accelerations but choose not to do so in step tracking movements. This may reflect different cerebellar contributions to the control mechanisms for generation of step and alternating movements.

473.3

A COMMON STRATEGY FOR THE CONTROL OF THE INITIAL AGONIST BURST INDEPENDENT OF MOVEMENT TYPE. J.D.Cooke & S.H.Brown (SPON: J.D.Brown), Dept. of Physiology, Univ. Western Ontario, London, Canada

We have tested the hypothesis that the desired movement acceleration and deceleration characteristics determine the properties of the phasic muscle activation driving movement.

Normal human subjects performed the following movements about the elbow: 1) step tracking movements of different amplitudes and durations, 2) cyclic movements of different amplitudes and frequencies, 3) phase plane tracking movements of different acceleration durations and 4) cyclic isometric movements of different frequencies and peak forces.

EMG burst durations and acceleration durations ranged from 100 to 500 ms. For each movement type the duration of the phasic EMG activity initiating movement varied linearly with acceleration duration. Across all movements/subjects the slope of the regression line was approximately 1.

The data show that relations exist between muscle activation and movement properties which are independent of movement type. Thus, the CNS can utilize knowledge of the desired acceleration duration in determining the appropriate duration of phasic muscle activation to initiate movement.

473.4

ARE SMALL AMPLITUDE MOVEMENTS CONTROLLED DIFFERENTLY FROM LARGE AMPLITUDE MOVEMENTS? H.Hefter*, J.D.Cooke, S.H.Brown, H.-J.Freund . Neurologische Klinik, Univ. Dusseldorf, Dusseldorf, FRG.

Very small amplitude movements (SAMs) are made with a sub-population of motor units. In addition, length and velocity dependent forces are of less importance than in faster, larger amplitude movements (LAMs). Do such differences lead to differences in the control of large and small movements?

Seven normal human subjects performed step tracking movements about the elbow with amplitudes from 70 to less than 2 deg. SAMs had mean velocities of less than 5 deg/s and peak velocities of less than 10 deg/s. Nonetheless, similar phasic EMG patterns occurred at every movement amplitude although not all the elements were present in every movement. The relation between peak velocity and movement amplitude was linear ($r > 0.9$). The relative durations of acceleration and deceleration and the ratio of peak to mean velocities did not vary systematically with movement amplitude.

In spite of the above mentioned differences in SAMs and LAMs, muscle activation patterns and movement kinematics were preserved across all movements indicating a common mode of movement generation.

473.5

MOVEMENT PLAN PREPARATION IS IMPAIRED IN ELDERLY PERSONS WHO FALL. PC Amrhein* and JC Morris. Department of Neurology, Washington Univ. Sch. of Med., St. Louis, MO 63110.

To determine whether community-dwelling elderly fallers (N = 15; mean age ± SD = 75.7 ± 6.6 y) differ from elderly nonfallers (N = 12; mean age = 75.0 ± 4.8 y) in speeded cognitive processes underlying preparation and execution of motor plans, both subject groups were assessed with respect to upper extremity movement for Reaction Time (RT) and Movement Time (MT). A paradigm was used in which 75% of the trials had valid precue stimuli (i.e., identical to target stimuli) but the remaining 25% had invalid precue stimuli (i.e., differed from target stimuli), thus necessitating reprogramming of the prepared response. Trials consisted of one of four precue stimuli presented on a computer screen for 200 msec, followed by a preparation interval of 875-1000 msec, and then four target stimuli to which subjects responded by manually pressing a corresponding button. Fallers had slower mean total (RT + MT) performance times for valid precues compared with nonfallers (1041 msec vs. 1016 msec, respectively), suggesting a lack of benefit for motor planning from the precue in fallers, but demonstrated faster mean total times for invalid precues (1138 msec compared with 1183 msec for nonfallers), implying that the motor plans of fallers required less alteration, presumably because they were less well established. The interaction between subject group and precue stimuli was significant ($p < .05$). We interpret these results to indicate that fallers have reduced cognitive ability to use relevant contextual information in the planning of simple movement tasks.

Supported by NIH Grant AG06815

473.6

PREPARATORY POSTURAL ADJUSTMENTS IN OLDER ADULTS AS A FUNCTION OF A BIMANUAL TASK. G.E. Stelmach, F. Müller and L.C. Populin*. Motor Behavior Laboratory, University of Wisconsin-Madison, Madison, WI 53706.

It is well-documented that older adults are slower than young adults, particularly in performing tasks that require the selection of a given movement over another, that is, Choice Reaction Time (CRT) tasks. According to Welford (1977), this response slowing observed under conditions of uncertainty, is due to the requirement to select a particular movement over another, and not simply due to slower movement execution.

The present study was designed to test this hypothesis by using a bimanual "push or pull" task. Eight older subjects (70-80 years) and 8 young subjects (20-24 years), all healthy and free of neurological disease, participated in the study. Subjects were required to react to a visual stimulus as fast as possible. Measures of response latency as well as EMG onset of relevant muscles were taken.

Results obtained showed that the older group selectively activated the tibialis anterior or gastrocnemius muscles (primary ankle stabilizers for push and pull movements, respectively) with the same latency as the younger subjects. Despite the similar muscle activation times, older subjects showed considerably longer movement latencies with increased response uncertainty. These results suggest that the slower response times observed in the uncertainty conditions were not due to processing deficiencies in determining the movement, but rather to deficiencies in the planning and/or implementation of the motor action. Additionally, the older group showed a differential pattern of activation of the musculature associated with anticipatory postural adjustments. Such differences may also have a detrimental effect on the implementation of voluntary movement in aging populations.

473.7

AN INVESTIGATION OF POSTURAL CONTROL STRATEGIES: THE INFLUENCE OF MATURATION. E.M. Earl* and J.S. Frank. Dept. of Kinesiology, University of Waterloo, Waterloo, Ontario, Canada N2L 3G1.

Research into the control of human posture has shown that consistencies are present in the responses adults make to compensate for internal and external perturbations (Cordo and Nashner, 1982). The purpose of this study was to investigate the influence of maturation on the reactive and anticipatory control of posture. Children, 4-8 years of age (n=17), were asked to perform voluntary handle pulls, and to resist externally generated handle translations. EMG and kinetic data were collected to document the compensatory adjustments used to cope with these disturbances. Maturation was found to enhance postural control. The frequency of anticipatory muscle activation increased as development progressed. Maturation was also associated with shorter latencies, and improved temporal coupling of the postural muscles during compensation for both types of perturbations. Although the children used a variety of muscle synergies to cope with both types of disturbances, the horizontal ground reaction forces were found to be consistent for both the internal and external perturbations. (Supported by NSERC)

473.8

ANTICIPATORY POSTURAL ADJUSTMENTS DURING INFANT REACHING. C. von Hofsten* and M.H. Woollacott. Dept. of Psychology, Univ. of Umea, Umea, Sweden and Inst. of Neuroscience & Dept. of PE and Human Movement Studies, Univ. of Oregon, Eugene, OR 97403.

Research on the execution of voluntary movements in humans has shown that anticipatory postural adjustments precede the forthcoming movement and thus compensate in advance for changes in equilibrium caused by the movement. It is possible that incoordination in reaching in young infants is caused by immaturity of the system involved in producing these anticipatory adjustments. To test the extent to which infants are able to use anticipatory activation of postural muscles of the trunk we performed the following experiments with 5 infants 9-10 months of age.

Surface Electromyograms recorded the activity of trunk extensor (TE), abdominal (A) and arm muscle (deltoid) responses, and a Selspot movement analysis system recorded hand movements as infants reached forward for a moving target while sitting on the parent's knee, supported only at the hip. All of the 5 infants showed anticipatory activation of the TE muscles before the deltoid on the majority of trials, thus stabilizing the trunk before the reach. However, responses were not present on all trials. This indicates that the system is functional, but possibly not mature by 9-10 months of age.

CONTROL OF POSTURE AND MOVEMENT IX

474.1

THE EFFECT OF COGNITIVE SET ON LONG-LOOP RESPONSES IN NORMAL SUBJECTS. D.J. Beckley*, M.P. Remler (SPON: J. Lieberman) Neurophysiology Lab, Dept of Neurology, VAMC; Martinez, CA 94553.

We investigated the influence of cognitive set on long-loop responses in normals subjected to rotational perturbations of various amplitudes. EMG recordings were obtained from 3 muscles in the left leg in 10 normal adults (age range = 25-41, mean 34.3; 5 females, 5 males). Stimuli consisted of three sets of 20 trials of sudden toe-up ramp movements of pre-selected amplitudes (pure 4°, pure 10°, and mixed 4°/10°) at a constant velocity of 35 degrees/S.Latencies for the short, middle and long-latency responses showed no significant differences. Mean normalized long-latency tibilias anterior (TA) amplitudes of both the pure and random 10° displacements were increased in comparison to the pure 4° displacements. Random 4° TA mean amplitudes were similar to random and pure 10° responses. We concluded that scaling of the amplitude of the TA long-latency response, and not changes in response latencies, represents the major postural adjustment mechanism in this paradigm and favors a large response in situations where perturbation size cannot be reliably predicted in advance.

474.2

FIRING ORDER OF MOTOR NEURONES DURING VOLUNTARY AND REFLEX ACTIVATION: **L.Davies*, A.W.Wiegner and R.R.Young. Spinal Cord Injury Unit. Veterans Administration Medical Center. Boston MA. 02132.**

The activation of motor neurones in a muscle pool is said to proceed as an ordered array in response to both increasing dorsal root stimulation and voluntary activation, with small motoneurones being recruited before larger ones. We have examined 19 voluntarily recruited soleus motor units in 5 human subjects and found that in 18 cases the lowest threshold motor unit recruited by voluntary activity is different from the lowest threshold unit recruited by electrical stimulation of the tibial nerve (Hoffman reflex). These low level voluntary units are, however, influenced by the stimulated fibres as, during tonic activation, their firing interval responds to a smaller stimulus than that required for H recruitment at rest. These findings suggest that the pool of soleus motoneurones responding to the voluntary command "plantar flex your ankle" differs, in order of activation, from the pool responding to stimulation of the largest diameter fibers in the tibial nerve . This could be explained by partitioning of excitatory input to the soleus motoneurone pool: either of cortical input or of Ia afferents.

474.3

FAILURE OF BALLISTIC MOTOR PROGRAMS TO COMPENSATE FOR UNEXPECTED TORQUE PERTURBATIONS: A.W.Wiegner and L.Davies*(SPON:J.Bossom). Spinal Cord Injury Unit. V.A.M.C. Boston, MA 02132.

During ballistic movements there is too little time for conscious modification of motor programs in response to proprioceptive input. We examined rapid flexion movements of the elbow for evidence of reflex activation in response to unexpected extensor torques during the movement.

Trained, rapid (peak velocity 467±76 deg/sec Mean±SD), accurate, flexion movements were interrupted by an opposing 6 Nm torque in 8 subjects who were unaware of such a possibility. During training, subjects developed an EMG signature characteristic for the movement. The earliest change in this signature after a torque pulse occurred at a mean of 248 ms which is similar to voluntary reaction time. Some subjects reproduced their entire movement signature, including inappropriate triceps activation, despite movement reversal by the torque. No evidence of a short latency compensatory response was seen. These findings suggest that in naive subjects there is no short-latency mechanism for modification of inappropriate ballistic movements once they are initiated.

474.4

THE EFFECT OF PRACTICE ON THE TIME TO PEAK AMPLITUDE OF SELECTED POSTURAL MUSCLES. C.S. Layne and D. Schnittker, Dept. of Physical Education, Kansas State University, Manhattan, KS 66506

A number of investigators have assessed the effects of practice on a variety of EMG variables. There are few studies designed to identify possible changes resulting from practice in the anticipatory postural patterns (APAs) observed during movement. It has been argued that invariant relationships between spatial and temporal muscle onset components support the claim that APAs and agonist activity may be controlled singularly (Lee, J. Mtr. Beh. 12: 3, 1980). The stability of the relationships between agonist and postural muscles' time to peak amplitude latencies (TPAs) have not been investigated. Five subjects performed 400 arm raises and the myoelectric activity of the biceps femoris (BF), paraspinals (PS) and anterior deltoid (AD) of the right side was monitored with surface electrodes. Trials 1-10 (Day 1) and Trials 391-400 (Day 3) were individually analyzed by obtaining the TPA for each muscle. Individual TPA means for each subject, for each day were computed. Ratios between TPAs were developed with t tests and F ratios being utilized to determine if practice effects were evident. Four of the five subjects displayed no change in their mean TPAs while TPA ratios were unaffected by practice. Mean TPAs failed to display a clear tendency toward less variability. While the TPA mean ratios remained unchanged with practice, the large variability on both Days 1 and 3 indicate that invariant relationships did not exist and suggests that TPAs are not centrally programmed.

474.5

EFFECTS OF LOAD-CARRYING ON MUSCLE ACTIVATIONS AND MOVEMENTS OF THE LOWER EXTREMITY DURING STAIR ASCENT. H. Moffet*, C.L. Richards and F. Malouin. Neurobiology Lab. and Dept. of Physiotherapy, Fac. of Med., Laval University, Quebec, Canada.

We studied the effects of load-carrying (with a weighted jacket) on muscle activations and movements of the right leg during stair ascent. Six normal subjects, aged 25 to 47 years, performed 3 tests consecutively: stair ascent 1) unloaded, 2) with a 16 Kg load, 3) with a 22 Kg load. During tests, sagittal movements (TRIAX electrogoniometer) and EMG activity (surface electrodes) from 5 muscles (vastus medialis: VM; vastus lateralis; medial hamstrings; medial gastrocnemius; tibial anterior) were recorded. Temporal parameters of the stair ascent cycle (cycle duration, % stance and swing) were measured from footswitch closures. Angle, EMG (amplified, rectified and time-averaged) and footswitch signals were simultaneously recorded (100 Hz) then fed to an IBM PC computer for analysis. Load-carrying (16 and 22 Kg) induced significantly higher EMG activity in the VM (area under activity profile from 0-20% of the cycle; ANOVA: $p<0.05$) during early stance of stair ascent. In contrast, the activation profiles of the other muscles, the movement profiles and temporal parameters did not change. The EMG increase in the VM for the two loading conditions was similar (19% and 20%) and largely exceeded the 1% variation computed for test-retest without loading. This selective response to loading in the VM during a critical period (0-20%) of the stair ascent cycle suggests that load-carrying during stair ascent may be a task specific test for VM function. This work was supported by a grant from Fonds de la Recherche en Santé du Québec (FRSQ).

474.6

EFFECTS OF STANCE WIDTH AND DISPLACEMENT SIZE ON AUTOMATIC POSTURAL RESPONSES TO LATERAL SURFACE DISPLACEMENTS. F.B. Horak, S.P.Moore, and J.M.Macpherson. R.S.Dow Neurological Sciences Inst. of Good Samaritan Hosp., Portland, OR 97209; Dept. of Anatomy, Queen's Univ., Kingston, ONT, K7L3N6.

The task of maintaining equilibrium in bipedal stance becomes more difficult as a function of the size of center of mass displacement relative to the width of the base of foot support. In this study, we examined the effects of stance width and perturbation size on kinematics and on medium latency EMG patterns in response to support surface displacements in the lateral direction. As stance width decreased or displacement size increased, subjects used more trunk lateral flexion with hip abduction/adduction to correct posture. These kinematic changes from an erect trunk to a flexed trunk were associated with an increased magnitude of EMG bursts but no changes in EMG temporal patterns or onsets. Reciprocally activated proximal trunk and hip muscles were always activated prior to coactivated distal ankle muscles. The magnitude of initial EMG bursts in both proximal and distal muscles were scaled to the size of surface displacements although bursts were initiated prior to the time information on displacement size was available to the CNS. These results indicate that as the postural task becomes more difficult, trunk lateral flexion increases due to increased muscle burst magnitudes based on current stance width and on prior experience with displacement size, without a change in the EMG pattern. (Supported by CIDA NS1094, ROl AG06457, and MRC of Canada.)

474.7

FREQUENCY AND VELOCITY CHARACTERISTICS OF HEAD, NECK, AND TRUNK DURING NORMAL LOCOMOTION. E. A. Keshner and B. W. Peterson. Dept. of Physiology, Northwestern Univ. Med. School, and Sensory-Motor Performance Program, Rehabilitation Institute of Chicago, Chicago, IL 60611.

The inertial mass of the head atop the flexible spine presents a physical system ripe for mechanical resonance and instability. A previous study of head stability while locomoting in place (Grossman et al., 1988), found that the head rotates at a frequency of 1.8 Hz in pitch and 0.8 Hz in yaw. Seated horizontal rotations (Keshner and Peterson, 1988) has demonstrated that head stabilization in space was excellent at low frequencies (< 1 Hz) as a result of voluntary mechanisms, and reached a resonant peak at about 2 Hz. The 1-2 Hz range might contain a crossover frequency at which a transition from neurally-dominated to inertially-dominated head stabilization occurs. To further determine the relative role of reflex and mechanical processes in head stabilization, this study investigated interactive dynamics of head, neck and trunk during normal locomotion. Pitch, roll, and yaw rotations were recorded from single axis velocity transducers placed on the head and trunk of three normal subjects (23-39 years) during walking along a 20 foot walkway at normal, slow, and fast speeds, running, and walking and running in place. Bipedal footswitches recorded heel strike onset. In all subjects, neck rotations most closely reflected head rotations. Mean velocity measures indicated that greatest velocities occurred in pitch. Velocities approximately doubled between fast and slow walking in all three planes, with small standard deviations between subjects. A power spectrum analysis revealed that responses in yaw were predominantly < 1 Hz at all speeds. Roll was a weak response with negligible responses of trunk, while head and neck responded most strongly at 1.5 Hz. Pitch generally produced head and neck responses at three frequencies: 1.5 Hz, 3 Hz, and 4.5 Hz, although preferred frequencies of rotation differed somewhat among subjects. Increasing speed of locomotion generally increased gains without changing frequency characteristics. Two resonant frequencies of the head could reflect the two primary axes of rotation in the neck. Different power spectra suggest that stability of head is controlled independently of the trunk.

Supported by grant NS22490.

474.8

INHIBITION OF AN HOMONYMOUS MONOSYNAPTIC, BUT NOT AN HETERONYMOUS OLIGOSYNAPTIC, SHORT LATENCY REFLEX IN THE HUMAN LEG DURING WALKING. D.F. Collins*, J.D. Brooke, and W.E. McIlroy. School of Human Biology and Biophysics Group, University of Guelph, Ontario, NIG 2W1, Canada.

Short latency reflexes are modulated by the level of ongoing contraction in the target muscle. However, at similar levels of contraction, Soleus (Sol) H reflexes are depressed during walking, compared to standing. Currently, we compared this movement modulation of a homonymous monosynaptic reflex to that of a previously identified heteronymous oligosynaptic reflex which involves two joints of the limb. H-reflexes were evoked in the Sol muscle during walking and standing, at equal stimulation intensities (as % Mmax in Sol) and over a range of contraction levels. The H Reflex magnitude was significantly depressed in the walking condition, compared to standing, at similar contraction levels. Oligosynaptic reflex excitation was evoked in the vastus medialis (VM) muscle by stimulation of the common peroneal nerve at caput fibula. No significant difference was identified between the VM reflex magnitudes during walking and during standing at equal stimulation intensities (as % Mmax in the tibialis anterior muscle) over a range of contraction levels. This complements the similarity of the VM reflex when pedalling versus sitting. The selective inhibition of the monosynaptic reflex exposes differences in the central control of these two segmental pathways during such movements. Supported by grant from NSERC Canada #A0025.

474.9

THE CONDITIONING EFFECTS OF CUTANEOUS STIMULATION ON THE HUMAN SOLEUS H-REFLEX DURING STANDING AND WALKING.

J. Fung and H. Barbeau. School of Physical & Occupational Therapy, McGill Univ. Montreal, Quebec H3G 1Y5

The modulation of the soleus H-reflex by a conditioning cutaneous stimulation was investigated in normal subjects during standing (n=5) and treadmill walking (n=2). The test H-reflex was obtained by stimulating the tibial nerve in the popliteal fossa using a single 1 ms pulse. The intensity corresponding to a maximum and stable H-reflex was first determined in standing. During walking, the effective stimulus strength was controlled by varying the intensity to obtain a stable M response similar to that observed in standing. The conditioning stimulus, consisting of an 11 ms train of three 1 ms pulses at 200 Hz, was delivered to the sole of the foot. The intensity was varied from sensory threshold (1T) to maximal (max T), with the flexion reflex in the tibialis anterior muscle monitored .

During standing, conditioning-test delays between 15 to 100 ms were given randomly. A conditioning cutaneous stimulation of max T resulted in a marked inhibition of the H-reflex amplitude (0% - 20% control) at 45 ms delay, and a late facilitation (200% - 300% control) at 100 ms delay. The H-reflex was not modulated with a conditioning cutaneous stimulation of 1T.

During walking, when tibial nerve stimulation was given alone, the H-reflex was modulated throughout the gait cycle, such that the amplitude was increased from midstance to push off, and inhibited in swing phase. When a conditioning cutaneous stimulation of varying intensity (1T, 1.5T, 2T, max T) was given 45 ms preceding the tibial stimulation, a progressive trend of inhibition was observed in midstance and push off, ranging from 100% of the control H-reflex amplitude with 1T stimulation, to a complete inhibition (0%) with max T stimulation. However, the H-reflex in the swing phase remained completely inhibited for all intensities.

These results confirm previous observations on the task-dependent modulation of the H-reflex, and further suggest that the increase in H-reflex amplitude during the stance phase of gait can be progressively reversed by a conditioning cutaneous stimulation of increasing intensity. (Supported by the MRC)

474.10

ADAPTING THE LOCOMOTOR PATTERNS TO CLEAR OBSTACLES. A.E. Patla, S. Prentice* and C. Robinson*. Dept. of Kinesiology, University of Waterloo, Waterloo, Ont., Canada, N2L 3G1.

The strategies adopted to clear obstacles (Al. plates raised by activating solenoids) of two heights (Low,High) when the visual cues about these obstacles were given at different times (Early & Normal-2 step and 1 step ahead) are examined. EMG signals from ipsi- (biceps fem.-IBF, rect. fem.-IRF, tib. ant.-ITA) and contralateral (biceps fem.-CBF, rect. fem.-CRF, soleus-CSO) muscles, vertical and anterior-posterior forces, and foot mat signals for 10 trials were collected for 4 experimental conditions (LN,HN,LE,HE), and 20 control trials. All trials were randomized and also videotaped. The results revealed a clear task dependent strategy. To clear the obstacles subjects slowed down and raised the center of mass (by ↑the vertical impulse). The significant muscle responses divided up into three functional phases, were as follows: 30-50%-↑IBF for HE condition, 50-70%-↑IBF for HE and HN conditions and ↑ITA for LN and HN cond.; 80-100%-↓IBF for the HE cond., ↑IRF for LN cond., and ↑CBF for LN, HN and HE conds. The effects of obstacle location within the stride were evaluated. (Supported by NSERC.)

474.11

A KINEMATIC ANALYSIS OF PERTURBATED LANDINGS. H. Sveistrup* and T.B. Hoshizaki (SPON: B. McFadden). Biomechanics Lab., McGill University, Montreal, Quebec, Canada, H2W 1S4.

Two women were unexpectedly dropped from a height of 18 cm and exposed to an 8 cm backward perturbation of the support platform at different delays following landing. The perturbation conditions were none, short delay (0.1 s), medium delay (0.2 to 0.3 s), and long delay (0.4 to 0.5 s). Cine data were collected (100 Hz) and lower limb joint angular kinematics evaluated. Pearson product-moment correlation coefficients calculated between trials within conditions were used to determine consistency in the shapes of the joint angular response curves. The range of the correlation coefficients collapsed across conditions for subject 1 was 0.80 to 0.99 for the ankle, 0.84 to 0.99 for the knee and 0.00 to 0.99 for the hip joint. The range of correlation coefficients for subject 2 was 0.88 to 0.98 for the ankle, 0.60 to 0.99 for the knee and 0.13 to 0.98 for the hip joint. For both subjects, the ankle and knee joints displayed consistent kinematic patterns while the hip joint was more variable indicating that the kinematic patterns remained fairly stable even though different neuromuscular strategies may have been used in response to the different task demands.

H. Sveistrup is partially funded by FCAR, Quebec.

474.12

NONSTATIONARY PROPERTIES OF POSTURAL SWAY. W. Freedman[1,2] and J.R. Carroll[3] 1.Drexel Univ., 2.Pennsylvania Coll of Optom., 3.Moss Rehab. Hosp., Plila. PA

When we stand, our bodies sway and are controlled back to the upright position in a stochastic manner. The postural sway process is measured by monitoring the output of a force-measuring substrate. There are a variety of statistics which are used to analyze the resulting center of pressure (COP) data, i.e., variance, RMS value of COP, path length, or sway area.

It is almost always implicitly assumed that postural sway is a stationary process in that the statistics do not vary over time. Although some investigators have discarded initial portions of their data before analysis where trends were obvious, no carefu study of this or of higher order moments exists.

In this study, we examine the time-invariance of the first and second order moments of postural sway and discuss the implications for sway measurement. We have measured sway on three normal individuals under conditions of quiet stance on two feet and one foot with eyes open and two feet eyes closed. Over many repetitions of the test (each one minute long), we collectec 51 minutes of data for each condition and for each subject.

The results show that there are trends in the postural sway data and transients in the second-order moments about the trends. The implications are that the time interval for data analysis must be chosen with care.

474.13

Ipsilateral associated torques measured during static effort at a single joint of the lower limb in normal subjects. D. Bourbonnais, J. Filiatrault*, D. Gravel*, A.B. Arsenault, M. Goyette*. School of Rehabilitation, University of Montreal and Montreal Rehabilitation Institute, Montreal Canada, H3S-2J4.

The effects of a static contraction on torques generated at an adjacent joint was investigated in normals. A dynamometer was used to measure isometric torques exerted simultaneously in flexion/extension, abduction/adduction and internal/external rotation at the hip and flexion/extension at the knee. This dynamometer was interfaced with a desktop computer which was used to display the direction and magnitude of subject's effort at a specific joint.

Subjects (n=4) were required to successively exert flexion and extension of the hip or the knee at 30% of the maximal voluntary contraction (MVC). Subjects were asked to perform flexion and extension of the hip without feedback regarding torques in abduction/adduction and internal/external rotation of the hip and torques generated at the knee. Similarly, flexion/extension of the knee was controled while no feedback was available on torques exerted at the hip.

Results show that subjects perform extension of the hip at 30% of MVC with an associated flexor torque at the knee probably reflecting the use of the hamstring muscles. In contrast, either flexor or extensor torques at the knee are observed with hip flexion. A flexor torque exerted at the knee results in a flexor, abductor and internal rotator torques at the hip. Extension of the knee causes a substantial extensor torque at the hip among all subjects evaluated.

It appears that associated torques are present in normals and that theses torques are greater during the production of a distal torque at a uniaxial joint than during execution of a proximal torque at a multi-axial joint. Our intent is to quantify and compare these associated torques in both normal and hemiparetic subjects. (Funded by Health and Welfare Canada MRC and FRSQ)

474.14

INFLUENCE OF GAIT PHASES ON CORTICALLY ELICITED MOTOR EVOKED POTENTIALS, J. Schild*, M.R. Dimitrijevic, R. Kinalski* and W.B. McKay*, Baylor Coll. Med. Houston, TX 77030.

By studying H-reflex amplitudes during gait, the excitability of the triceps surae spinal motor pool has been shown to increase in stance and decrease in swing phase. Motor evoked potentials (MEPs) elicited by transcranial stimulation of the motor cortex can also be used to demonstrate such excitability changes in gait, since it is known that MEPs are increased in amplitude by voluntary activity in target muscles. For this purpose, we employed a Cadwell magnetic stimulator applied over the vertex. Surface EMG responses were recorded from lower extremity muscles including quadriceps, hamstrings, tibialis anterior and triceps surae muscles. Stimuli were delivered at mid-stance and mid-swing by varying the stimulus delay after heel strike. With near-threshold stimuli, in the stance phase of gait, the response in the soleus was larger than in the tibialis anterior. In the swing phase, just the reverse was true. When higher stimulus levels were used, the dependence of the response amplitudes on gait cycle was lost. The lower-intensity findings reveal an antagonistic, dynamic variation in excitability dependent on the phase of the gait cycle. However, by activating a larger population of descending axons, this variation in the MEPs was overcome, even to the extent of disrupting ambulation. This may be due to the effects of the size of the excited population of a potent descending system.

474.15

TEMPORAL CHARACTERISTICS OF ANKLE MUSCLE ACTIVITY DURING THE INITIATION OF STEPPING IN HUMANS. M.W. Rogers* and S.I. Lin*(SPON:H.Davis).Physical Therapy,Northwestern Univ. Med. Sch.,Chicago, IL 60611.

The transition from stationary standing to walking is initiated via a deactivation of the ankle extensor muscles followed by an activation of the ankle flexors, which presumably elicits and reinforces a forward acceleration of the body center of mass prior to the removal of the stepping leg from the ground.

The ankle muscle timing pattern was examined prior to heel-off(HO) of the initial step with respect to possible differences in control strategies for self-paced(SP) vs. externally triggered(ET) movements, and in interlimb responses related to upcoming swing and stance functions. Subjects(n=5) stood on a force platform and executed a series of steps under ET(light-flash) and SP conditions. Surface EMG was recorded from soleus(SOL) and tibialis anterior(TA) muscles bilaterally, while a foot switch detected the initial HO. Three principal findings emerged:(1)An offset of tonic SOL activity always($p<.05$) preceded TA activation;(2)A generally earlier mean offset(SOL) or onset (TA) for SP vs. ET movements at comparable stepping speeds was observed, while intersubject variability was considerably greater for the former;(3)The intermuscular timing interval(SOL-TA) was longer($p<.05$) for the upcoming stance vs. swing leg, and may reflect the need to unload the latter while continuing the forward acceleration of the body.

474.16

CHANGES IN JOINT POSTURE AND CENTER OF MASS LOCATION DURING ABRUPT PULLS OF DIFFERENT FORCES MADE BY STANDING HUMANS. W.A. Lee, C.F. Michaels* & Y.C. Pai*, Physical Therapy, Northwestern University, Chicago IL 60611.

Bernstein (1967) hypothesized that the control of complex actions is simplified by the adoption of invariant movement synergies that are scaled to the speed or force of the action. Alternatively, different movement patterns might be used for actions with different dynamics. The purpose of our study was to determine if a single movement synergy characterizes bilaterally symmetrical, abrupt pulls in the sagittal plane made by well-practiced standing humans.

Hip, knee and ankle joint angles, and the locations and velocities of the center of mass (CM) in the anterior/posterior (a/p) and vertical (v) directions were computed from data collected with a WATSMART system from 3 well-practiced subjects who pulled at 5, 10, 20, 40, 60, 80 and 95% of maximal pulling force (%MPF). Overlaid time records, angle-angle diagrams and CMa/p vs CMv plots were used to determine if movement patterns were invariant. Linear regressions assessed the extent to which the peak change in each variable was scaled to %MPF.

The onset, peak, and offset times of changes in each variable were relatively constant. Only the peak change in CMa/p, CMa/p velocity and ankle angle were significantly correlated with %MPF in all three subjects. Little or no change occurred in any variable at 5 or 10% MPF. All subjects' CMa/p moved backward before pulls $\geq$20%MPF. CMv decreased before pulls > 40%MPF for Subj. 1 and 2, but only before 95%MPF pulls for Subj. 3. Subj 1 rotated back, plantarflexing the ankles and flexing the knees and hips before 10-20%MPF pulls; $\geq$40% MPF, she made a coordinated movement of ankle dorsiflexion, knee and hip flexion followed by ankle plantarflexion (other angles constant), ending with hip and knee extension ("drop and thrust up"). Subj. 2 plantarflexed only the ankles before 10-20% MPF pulls ("rotate back, body straight"); concurrently plantarflexed the ankles and flexed the hips at 40-80% MPF ("jack-knife back"); and flexed all three joints at 95%MPF. Subj. 3's backward movements occurred almost exclusively at the ankle , but some concurrent hip and knee flexion occurred above 60%MPF.

We conclude that subjects alter their movement patterns as pulling force increases, which argues against the hypothesis that one movement synergy underlies bilaterally symmetrical abrupt pulls. The variations in knee, hip and ankle angle and CM movement patterns shows that subjects have some flexibility in organizing the kinematic degrees of freedom of the task.

474.17

EMG TURNS ANALYSIS REVISITED. D. Junge. Dental Res. Institute, Sch. of Dentistry and Dept. of Physiology, Univ. of Calif., Los Angeles, CA 90024.

"Turns," or reversals of direction of more than 50 or 100 μV, were studied by digital analysis of surface EMG records from masseter muscles. The purpose was to compare this method with rectification and smoothing as quantitative estimates of multiunit interference patterns. Inter-turn interval distributions were skewed toward shorter intervals, with means between 3 and 10 msec at various percentages of maximal contraction. The amplitude changes between turns had rather flat distributions ranging from 50 - 600 μV. When the signal gain was varied, both the number of turns / 0.5-sec sample period and the average inter-turn interval were less sensitive to gain than was the smoothed, rectified signal. The average interval between single motor-unit action potentials (MUAPs) in surface records at low levels of contraction was 47 msec. When 25 simulated MUAP trains with an average interval of 44.9 msec were added together, the mean inter-turn interval was 2.85 msec. Increasing the average interval between MUAPs to 96.9 msec caused the mean inter-turn interval to increase to 3.79 msec. Thus, the turns in surface EMG records appear sensitive to frequency of component MUAPs, but less sensitive to signal size than smoothed, rectified signals.
Supported by USPHS Grant # RR05304.

OCULOMOTOR SYSTEM IV

475.1

THE EFFECTS OF LESIONS OF THE SUPERIOR TEMPORAL POLYSENSORY AREA ON EYE MOVEMENTS IN THE MACAQUE. J.P. Skelly, T.D. Albright, H.R. Rodman, and C.G. Gross. Dept. of Psychology, Princeton University, Princeton, NJ, 08544.

The superior temporal polysensory area (STP) is a predominantly visual area located in the upper bank and fundus of the rostral superior temporal sulcus. On the basis of its anatomical connections and single unit response properties, we suggested that STP may play a role in visual orientation and eye movments (Bruce et al., J. Neurophysiol., 46:369, 1981). We have now studied the role of STP in oculomotor control by removing STP in animals trained to make saccadic and smooth pursuit eye movements to visual targets. Saccadic eye movements were made to targets that appeared 8, 15, and 22 degrees rightward and leftward from the fixation point and 8 degrees above and below the fixation. Smooth pursuit eye movments were made to targets moving 5, 13, and 20 degrees per second.

Three of four STP lesions resulted in a mean increase in saccade latency of 90 msec to the most peripheral contralateral target and smaller saccade latency increases to less peripheral targets. The unimpaired animal had the smallest lesion. Saccade latency recovered to the preoperative level in three to five weeks after surgery. Saccade accuracy was unimpaired by any STP lesion.

In contrast with the saccadic latency increase, STP lesions did not produce any smooth pursuit deficit. These results suggest that the saccade latency deficit does not reflect a general oculomotor or motivational deficit, but rather an impairment in visual orientation.

475.2

PURSUIT RECOVERY AFTER FRONTAL EYE FIELD LESIONS IN MONKEYS. J. C. Lynch. Departments of Anatomy and Ophthalmology, University of Mississippi Medical Center, Jackson, MS 39216

Recovery of visual pursuit following bilateral FEF lesions was studied in three macaque monkeys trained to track visual targets moving horizontally at 20, 30, or 40°/sec. Immediately after the lesions, slow eye movement gain fell to 0.1 - 0.5 and repeated corrective saccades were interposed between periods of slow eye movement. The mean velocity of combined slow and saccadic movements during a single trial provided a close match with the target velocities. Recovery proceeded at the same rate for all three target velocities, with the gain of the slow eye movements gradually returning to normal over a period of 2 to 6 weeks. Corrective saccade amplitude was relatively uniform during the course of recovery, ranging between 6° and 10° for different monkeys and different pursuit velocities.

These results suggest that monkeys with FEF lesions can accurately calculate the velocity of a moving target, but cannot generate the smooth eye movements appropriate to match the target movement. This deficit is therefore qualitatively different from the pursuit deficit which follows cortical lesions of the temporal and parietal areas MT and MST, in which the velocity of a pursuit target is no longer calculated accurately.

(EY-04159 and the Vaughan Stroke Research Trust.)

475.3

OCULOMOTOR DEFICITS ASSOCIATED WITH LESIONS OF THE FRONTAL EYE FIELD AREA IN MACAQUE MONKEYS. M.G. MacAvoy and C.J. Bruce. Sec. of Neuroanatomy, Yale Univ. Sch. of Medicine, New Haven, CT 06510.

Two adult rhesus monkeys were trained on a battery of saccadic and smooth pursuit oculomotor tasks and then received sequential unilateral FEF lesions. We aspirated the anterior bank of the arcuate sulcus, targeting the FEF as defined by Bruce et al. (J. Neurophysiol. 1985) on the basis of elicited saccades. Several months of testing followed each lesion. We conclude that this cortex participates in smooth pursuit, saccades to moving targets, and predictive eye movements, both saccadic and smooth.

Following the first lesion (right FEF) in one monkey and the second (left FEF) in the other, the gain of smooth pursuit was dramatically reduced for tracking in directions ipsilateral to the lesion, with contralateral pursuit unaffected. Subsequent to the lesions of the other hemisphere, however, smooth pursuit gain was unaffected. We think that the effective lesions included the restricted area in and near the fundus of the arcuate sulcus where microstimulation elicits smooth, usually ipsilaterally-directed, eye movements (MacAvoy, et al., Soc Neurosci Abstr, 1988), in contrast to contralateral saccades readily elicited from most of FEF.

We analyzed this smooth pursuit tracking deficit with sinusoidal, linear, and step-ramp tasks. Sinusoidal tracking was very asymmetric; all frequencies tested (0.25-2.5Hz) had reduced smooth pursuit velocity and a preponderance of catch-up saccades for the ipsilateral direction. Similar deficits obtained with linear tracking, with low gains even at low target velocities, even though smooth velocities up to 35°/sec were obtained with a 75°/sec target. This directional deficit was present in response to step-ramp target motion in both the left and right visual fields. Ipsilateral saccadic eye movement size and latency in conjunction with ramps were also affected.

Predictive aspects of oculomotor behavior were drastically affected, especially eye movements involving target motion ipsilateral to the effective lesion. Anticipatory smooth pursuit preceding centripetal target motion was almost abolished for ipsilateral directions. Predictive continuation of sinusoidal tracking following target extinction was similarly affected. Predictive saccadic tracking was also reduced. Supported by PHS grants EY04740, NS22807, MH44866.

475.4

SACCADES IN HUMANS WITH LESIONS OF FRONTAL EYE FIELD (FEF) W.A. Fletcher* and R.S. Gellman Depts. of Clin. Neurosci. and Med. Physiol., Univ. of Calgary, Calgary, AB, T2N 2T9.

We studied horizontal and vertical saccades in 10 patients with unilateral lesions of FEF and 10 age-matched normal subjects using predictable (PT), unpredictable (UT) and remembered targets (RT). Subjects also followed a target which alternated between two horizontal positions at progressively shorter intervals (2.0 - 0.1 sec; AT).

Mean saccadic latencies for UT and RT and saccadic accuracy and peak velocities for all target conditions were normal. However, saccadic latencies for PT were abnormal in 8 patients: 3 had prolonged latencies contralateral to the lesion, 1 had bilateral prolongation and 4 had subnormal latencies due to excessive predictive saccades. For AT, the minimum intersaccadic interval preceding contralateral saccades (group mean = 463 ± 143 msec) was greater than that preceding ipsilateral saccades (290 ± 134 msec; $p < 0.02$). During attempted vertical saccades to RT, horizontal components frequently caused oblique saccades toward the side of the lesion in 7 of 9 patients. The difference in mean horizontal component was significant ($p < 0.01$) when patients with left-sided and right-sided lesions were compared.

In humans, the FEF likely participates in triggering normal saccades and inhibiting inappropriate saccades. Both FEFs may be required to program purely vertical saccades.

475.5

UNIT ACTIVITY RELATED TO SMOOTH PURSUIT EYE MOVEMENTS IN RHESUS MONKEY FRONTAL EYE FIELDS J.P. Gottlieb*, M.G. MacAvoy and C.J. Bruce, (SPON: D. Burman) Section of Neuroanatomy, Yale University School of Medicine, New Haven, CT 06510

Single unit activity was studied within and near the region of the frontal eye fields (FEF) where smooth eye movements are elicited by microstimulation (MacAvoy et. al., Soc. Neurosci. Abstr. 1988). 44 neurons responded on linear (constant velocity) or sinusoidal smooth pursuit tracking tasks, but had no (or minimal) responses during visually-guided saccade tasks. All neurons were tuned for pursuit direction, and their responses began 60-90 ms after target movement onset. During linear tracking most neurons had tonic responses lasting throughout the track -- 2 s or longer; others responded for only 3-400 ms. Activity during sinusoidal tracking closely followed the periodicity of stimulus and eye motions. Most neurons were also tested while the monkeys fixated a stationary light during the presentation of sinusoidally moving stimuli. Response magnitude, duration, phase, and optimal direction were strikingly similar to those obtained for tracking of the same stimulus motion, despite the large reduction in retinal slip due to tracking. Many neurons also showed activity that could be interpreted as "anticipatory" or "predictive": 1) During ongoing sinusoidal tracking activity began before the stimulus (or the eye) reversed to the neuron's preferred direction. 2) If the sinusoidally moving target was extinguished immediately before reversing to the neuron's ON direction a response still followed, as if anticipating the reversal. 3) Anticipatory activity was observed up to 500 ms before motion commenced on trials on which the monkey could expect centripetal motion (linear or sinusoidal) in the unit's preferred direction. We conclude that neurons in the primate FEF are involved in the generation and control of smooth, as well as saccadic, eye movements. Supported by PHS grants EY04740 and MH44866.

475.6

FEEDBACK FROM NATURAL SACCADES PERTURBS THE DIRECTION AND AMPLITUDE OF SACCADES ELICITED BY MICROSTIMULATION IN THE MONKEY'S FRONTAL EYE FIELDS. Charles J. Bruce and Gary S. Russo. Sect. Neuroanatomy, Yale Univ. Sch. Med., New Haven, CT 06510.

Saccades are coded topographically across the primate frontal eye fields (FEF), with each location representing a particular direction and amplitude; however, Schlag and Schlag-Rey (1987, 1988) recently reported that the metrics of saccades electrically elicited from the FEF can be dramatically modified if the stimulation conicides with a natural saccade. We further examined this phenomenon, testing FEF sites yielding saccades at a low thresholds (< 50 μA). The monkey began each trial by fixating an eccentrically-located target. After ~1 s the target jumped to the center of the monitor, and the monkey's ensuing saccade automatically triggered a 70 ms train of electrical stimulation within ~10 ms after the saccade began. This stimulation usually elicited another saccade that began shortly after the natural one ended. By varying the location of the eccentric fixation target, we analyzed the effect of the dimensions of the natural saccades upon the dimensions and latencies of the electrically elicited ones. Our results confirm those of Schlag and Schlag-Rey in that the elicited saccades were systematically modified; however, saccade dimensions were not predicted simply by translating the retinotopic FEF vectors to craniotopic goals. Instead, we hypothesize that the modified elicited saccades reflect a partial vectorial subtraction of an efferent copy of the preceding natural saccade from the characteristic saccadic vector of the stimulation site. In the natural situation this subtraction serves to cancel FEF activity immediately after it is used, thereby preventing multiple saccades. Moreover, it also provides a non-"spatial" explanation for oculomotor behavior in the double-step paradigm of Hallet & Lightstone, and thereby alleviates much of the motive for postulating a craniotopic stage of saccadic processing. PHS Grants EY04740 & NS22807.

475.7

TOPOGRAPHICAL PROJECTIONS FROM THE FRONTAL EYE FIELDS TO PARIETAL, TEMPORAL AND OCCIPITAL CORTICAL AREAS IN THE MACAQUE MONKEY. G. B. Stanton, M. E. Goldberg, and C. J. Bruce. Dept. of Anatomy, Howard Univ. Coll. of Med., Washington, DC 20059, Lab. Sensorimotor Res., Natl. Eye Inst., Bethesda, MD 20205, and Section of Neuroanatomy, Yale School of Med., New Haven, CT 06510.

Frontal eye field (FEF) projections to posterior cerebral cortical areas were studied with autoradiography in four macaque monkeys. Injections of tritiated amino acids were placed into large (lFEF) and small (sFEF) saccade sites within the physiologically defined FEF. Labeling was usually densest and most widespread in layer I and moderately dense in layers V-VI with hot spots of labeling in all layers. Hot spots for lFEF projections were (caudal to rostral) the anterior lip of the posterior parietal lobule (PPL); superior bank of the superior temporal sulcus (STS) and posterior cingulate gyrus. Light labeling was seen on the superior lip of the calcarine sulcus. The lFEF labeling was probably in functional areas 7a/LIP, MST, peripheral V2 and the superior temporal polysensory area. Hot spots for sFEF projections were the anterior wall of the PPL and the floor and inferior bank of the STS. Light labeling was seen lateral to the occipitotemporal sulcus anteriorly, and along the medial wall of this sulcus and the inferior occipital sulcus posteriorly. Functional areas VIP, MT, FST, V2, V3a, V3d, V4t were probably labeled from sFEF. These results suggest that the FEF are topologically organized with respect to visual and visuomotor areas of the posterior cortical areas. Supported by NIH grant EY03763.

475.8

INTERACTIONS OF VISUAL AND MOTOR-PLANNING ACTIVITIES IN THE LATERAL INTRA-PARIETAL AREA (LIP). S. Barash, R.M. Bracewell, L. Fogassi*, and R.A. Andersen. Dept. of Brain and Cognitive Sciences, Rm E25-236, M.I.T., Cambridge, MA 02139.

We further investigate the role of macaque cortical area LIP in visual-motor integration.

I. Detailed quantitative analysis of 145 LIP neurons revealed: (1) Activity during delayed saccade trials typically has 3 distinct phases - light sensitive (LS), memory related to target location (M), and saccade-related (SR), beginning before or during the saccade. In contrast, 51 similarly tested area 7a neurons usually had weaker LS, M and SR activities, but strong post-saccadic activity. (2) LS and SR field widths [~100 deg at 50% peak activity] were similar to published figures for frontal eye fields. (3) The preferred directions of LS, SR and M were quite similar. (4) A subset of neurons, selected for their clear LS and SR responses and narrow (<90 deg) fields, had excellent alignment of the LS and SR preferred directions (median difference = 12 deg). Thus, an accurate linkage of visual and motor spatial representations exists in LIP.

II. (1) Using the double-saccade paradigm, we previously found that LIP cells are active before a second saccade made in the motor field of the cell, even without sensory stimulation in its receptive field. Thus, LIP cells code in motor coordinates. We now employed the complementary paradigm: flashing a target inside the receptive field, but requiring an intervening saccade in a non-preferred ("null") direction. In these conditions, cells remain silent until the completion of the 1st saccade, and only then become active in anticipation of the 2nd saccade. Thus the plan of the 2nd movement is not expressed in the activity before or during the 1st saccade. Furthermore, the visual response is contingent on the forthcoming saccade being planned into the cell's motor field.

(2) In a novel "change of motor plan" paradigm we mixed 4 classes of trials. Two classes are delayed saccades, in either the preferred or a null direction. In the other 2 classes the required saccade direction is changed midway through the delay period by flashing the alternative target. When the the null direction is replaced by the preferred direction, the cell only becomes active following the onset of the second target. Conversely, an elevated level of activity induced by a target in the preferred direction is clearly reduced by flashing a null target. Therefore, the activity of LIP cells represents mainly the next planned movement; and alterations of this plan, even without eye movements, are reflected in altered LIP cell activity.

475.9

MICROSTIMULATION OF A NEURAL NETWORK MODEL THAT COMPUTES COORDINATE TRANSFORMATIONS FOR VISUALLY GUIDED SACCADES. R.A. Andersen and S.J. Goodman*. Dept. Brain & Cog. Sci., M.I.T., Cambridge, MA 02139.

Visual stimuli are imaged on the retinas but eye movements to visual targets require commands that specify the locations of the eyes in the orbits; thus the programming of visually guided saccades requires a transformation from retinal to head-centered coordinates. We have trained a neural network to make such a conversion and have found that simulated electrical stimulation of elements of the network produces "eye movements" like those produced by stimulating saccade centers in the brain. At the input of our model are eye position and visual signals similar to those found in the posterior parietal cortex of macaques; the output is the frequency of firing code to a simplified group of extraocular muscles. The trained network produces simulated visual and saccade responses in the middle-layer units that exhibit the same type of eye-position dependencies as those recorded from the lateral intraparietal area (LIP) in the posterior parietal cortex.

When brain areas are microstimulated at different initial eye positions, three types of eye movements result: "fixed vector" saccades in which the same direction and amplitude eye movements are evoked regardless of initial eye position, "convergent" saccades in which the saccades converge to a single head centered location but rarely reverse direction, and "amplitude-change" saccades in which the amplitude of the saccade is reduced in size at more peripheral eye positions in the direction of the saccade. Once our model had been trained we "stimulated" it at different eye positions by saturating the activation of individual middle-layer units and found the network produced all three types of movement.

Interestingly, the visual receptive fields, motor fields, and gain fields all have the same best-direction in both the individual model units and in many area LIP neurons. Detailed examination of the network showed that the same best-direction characteristic of the three parameters accounted for the saccade behaviors of the network and similarly may account for the saccade behaviors produced by brain stimulation.

475.10

MICROSTIMULATION OF PRIMATE FRONTAL EYE FIELD SPECIFIES RETINOTOPIC GOAL OF EVOKED SACCADE. P. Dassonville, J. Schlag and M. Schlag-Rey. UCLA, BRI & Dept. of Anatomy, Los Angeles, CA 90024.

The colliding saccade paradigm, i.e., microstimulation during or shortly after an ongoing saccade, can differentiate the roles played by various oculomotor regions in saccade generation. Deep collicular stimulation specifies the saccade vector, whereas superficial collicular stimulation specifies the saccade retinotopic goal. Thalamic stimulation creates an artificial *retinal* error signal referred to a *previous* eye position, with a delay adequate for compensating early visual processing time (Exp. Brain Res., in press). Applied to the frontal eye field (FEF) in 2 monkeys (*Macaca nemestrina*), the paradigm shows that, as in the thalamus, microstimulation creates a retinal error signal referred to a previous eye position, eliciting a saccade compensatory for both afferent and efferent delays in the oculomotor system. Compensation was seen at 37 stimulation sites regardless of the type of presaccadic unit activity present, i.e., visual, visuomovement, or movement. This suggests that the output of the FEF represents a saccade goal in retinal coordinates, rather than a motor command. This goal is later translated into spatial coordinates. (Supported by USPHS grants EY05879 and EY02305, and NSF grant RCD87-58034).

475.11

THALAMIC CONNECTIONS OF MONKEY SUPPLEMENTARY EYE FIELD. B. Shook, M. Schlag-Rey and J. Schlag. Dept. Anatomy & Cell Biology, UCLA , Los Angeles, CA 900024.

WGA-HRP was injected into the supplementary eye field (SEF) defined by recording and stimulation. Thalamic connections were compared to the contiguous SMA, dorsal primary motor cortex(M1) and FEF. Thionin and Ache/CYO histochemistry were used to define thalamic boundaries.

SEF injections lead to dense anterograde and retrograde label in the ventral anterior (VA) complex and medialis dorsalis (MD). Connections were most robust in the medial aspects of VAmc and area X where these nuclei abut the internal medullary lamina. Dense reciprocal connections were seen in MDmf, lateral MDpc and tenuously, in the anterior pole of MD and MDpc. SMA deposits yielded dense terminal and somal label in lateral aspects of VApc and area X, and moderate reaction product was observed in ventrolateral nuclei. Sparse bidirectional label was found in ventral MDmf and in a discrete zone of MDpc. FEF injections produced a pattern of label very similar to SEF (VA, VApc, VAmc, X, MDmf, MDpc, MDdc) differing by an absence of orthograde label in VApc. M1 connections resembled SMA except for a dense reciprocal pathway with VPLo.

Injections yielded sparse label in the intralaminar nuclei (PCN and CL). SMA and M1 yielded ventral label and SEF/FEF dorsal label. SEF, FEF and SMA deposits labeled CSL. (USPHS Grants EY02305 & EY05879).

475.12

AUDITORY RECEPTIVE FIELDS OF NEURONS IN FRONTAL CORTEX OF RHESUS MONKEY SHIFT WITH DIRECTION OF GAZE. Gary S. Russo and Charles J. Bruce Sect. Neuroanatomy, Yale Univ. Sch. Med., New Haven, CT 06510.

Auditory receptive fields of single neurons within and near the frontal eye fields were mapped during visual fixation. White noise was delivered from one of an array of 8 speakers distributed along the horizontal meridian from -120° (far contralateral) to +90° (ipsilateral) and each speaker location was tested during each of 3 fixation positions (-28°, 0°, +28°) in pseudorandom order. Response magnitude was fit to Gaussian tuning curves to estimate each cell's best auditory direction and tuning width for each fixation condition.

During central (0°) fixation the median best auditory direction of 31 neurons was -18°. Best angles ranged from -110° to +10°, with 80% in the contralateral field, 10% in the ipsilateral field, and 10% straight ahead (0°). The median tuning curve half-width, estimated by the SD of the fit, was 25°. 7 of these neurons also responded to visual stimuli.

Eccentric fixation usually shifted the auditory tuning curve in the direction of gaze. For 27 neurons the median shift of best direction for ipsilateral fixation (+28°) was +9.2° whereas the median shift for contralateral fixation (-28°) was -8.7°. This shift was quantified for each neuron by the regression of best auditory direction on direction of gaze; the median auditory field moved ~0.325° per degree of gaze shift. Auditory field plots usually remained similar in width; however, gaze direction also significantly affected the overall auditory response magnitude of several cells.

Similar gaze-effected shifts of auditory fields occur in the superior colliculus (Jay & Sparks, Nature '84) intermediate layers which receive heavy projections from this frontal lobe region. These shifts could represent a partial transformation from craniotopic to retinotopic coding in the neural representation of auditory location. Such a transformation, if completed elsewhere in the brain, could be a neural basis for integration across sensory modalities and for targeting saccades to sounds. However, it is unclear to what extent this shift reflects central or peripheral mechanisms since rhesus monkeys typically orient their pinna towards their direction of gaze (Bruce, et al., Soc. Neurosci. Abstr. '88).

475.13

ARE SACCADE LATENCIES BASED ON THE CRANIOTOPIC LOCATION OF THE TARGET? R.J. Tusa and J.L Becker* Dept of Neurology, Johns Hopkins Hospital, 600 North Wolfe St., Balt. MD, 21205.

Latencies of saccade to targets unpredictably stepped to 1 of several positions from L20° to R20° were measured in 3 monkeys with eye coils. We found that latencies varied according to the orbital position of the saccade. In the table we show the latencies of rightward saccades to the 5° steps. Latencies increased as orbital position moved from extreme left gaze to right gaze. This relation appeared to be based on the craniotopic (head-centered) position of fixation and not the ending position. For example rightward saccades that started at L20° and ended at R20° had latencies similar to the 5° saccades generated from the same starting position and ending at R15°.

Step size	Lat (msec)
L20-L15	128±11
L15-L10	131±12
L10-L05	140±21
L05-00	148±18
00-R05	157±27
R05-R10	167±27
R10-R15	185±25
R15-R20	211±29

These results suggest that saccade latencies vary according to the craniotopic location of fixation. We are currently evaluating whether this effect is based on differences in time taken to shift visual spatial attention.

475.14

COMPONENT CROSSCOUPLING AND TRAJECTORY CURVATURE IN FAST AND SLOW HUMAN SACCADES. J.A.M. Van Gisbergen* and A.C. Smit* (SPON: European Neuroscience Association). Dept. of Med. Phys. & Biophys., 6525 EZ Nijmegen, The Netherlands.

Experimental data indicate that the dynamic properties of human oblique saccade components are not independent but show mutual crosscoupling (stretching) and that oblique saccade trajectories are often curved. In order to test whether existing 2D models for the control of saccades can account for these phenomena, we have investigated fast saccades to visual targets (V) and slower saccades to remembered targets (R) in 4 subjects using a magnetic field method.

We found that the relative amount of stretching was comparable in V- and R-saccades. Quantitative analysis of the saccade trajectory revealed periodic changes of curvature with saccade direction which were similar in both saccade types. From these data we conclude that the factors determining component stretching and trajectory curvature probably reside in the final common pathway for V- and R-saccades. A vectorial pulse generator model (Van Gisbergen et al., 1985) can explain the observed stretching phenomena quite well but cannot account for saccade curvature. A model with crosscoupled orthogonal pulse generators (Grossman and Robinson, 1988) yields reasonably accurate stretching but predicts too much curvature.

475.15

SACCADES IN PROGRESSIVE SUPRANUCLEAR PALSY (PSP)
W.T. Cornblath*, T.C. Hain, N.R. Miller (SPON W. Brownell). Depts of Neurology, Otolaryngology, and Ophthalmology, Johns Hopkins University, Baltimore MD, 21205.

PSP is a degenerative disease of humans in which most damage is found in the globus pallidus, subthalamic nucleus, pretectum and superior colliculus. In 3 subjects with PSP we measured saccades with the scleral eye coil.

Horizontal and vertical latencies were 242 (range 214-237) and 363 (279-426) msec for patients and 204 (181-231) and 212 (201-241) msec for normals. Saccades to 40 deg targets averaged 18.5 deg horizontally and 15.5 deg vertically. Horizontal saccade velocities declined from 95% of normal at 1 deg to 50% of normal at 10 deg. Vertical saccade velocities declined from 53% of normal at 1 deg to 20% of normal at 4 deg.

The difference in latencies implies separate initiating mechanisms for horizontal and vertical saccades. The inability to make large saccades and reduction in the velocity of saccades is similar to the enduring oculomotor deficit reported in monkeys with combined frontal and collicular lesions (Schiller et al, 1980). The normal speed of a sub-population of horizontal saccades implies that horizontal burst neurons are intact, which is consistent with the pathologic findings.

475.16

POST-SACCADIC DRIFT IN STRABISMICS FOLLOWING BOTULINUM INJECTION.
Z. Kapoula and L. Garraud*. Lab. Exper. Psychol. CNRS-UA 316, Paris; Douarnenez Hosp., Brittany, France.

We examined post-saccadic drift in four adult patients with esotropia (10-40 diopters) before and after injection of botulinum in the medial rectus of one eye. Saccades were recorded binocularly with the EOG; patients viewed binocularly horizontal LED targets in all sessions. After injection, the altered eye was patched. The toxin created changes in squint and marked, position-dependent, post-saccadic drift in that eye within one day. We concentrated on abducting saccades in the range of action of the non-altered muscle. The pre-post change in drift of the altered eye was 25, 7, 30% of the antecedent saccade for the patients examined 1 day later, and 87% for one patient examined 3 days later. After 4 hrs of binocular viewing, drift in the altered eye decreased by 2-10%; however, this induced drift in the non-altered eye by a similar amount (Hering's law). Three patients were re-examined 2 weeks later; two days before examination the injected eye was patched. Surprisingly, drift in the altered eye was still marked (39, 55, and 45%). After 5-7 hrs of binocular viewing, one of these patients decreased this drift by 13% with no changes in the other eye (monocular correction); the other two patients made a conjugate change of 7%. Note, the patients had no fused binocular vision before or during these experiments. These results suggest limits in making monocular corrections of drift created by paralysis of the injected muscle.

475.17

EXCESSIVE ANTICIPATORY SACCADIC EYE MOVEMENT IN SCHIZOPHRENIA.
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We examined smooth pursuit as well as purely saccadic movements in neuroleptic-treated schizophrenics (n=22), patients with mixed drug and alcohol abuse (n=20) and normals (n=17). Schizophrenics had slightly (but significantly) lower pursuit gain and made more saccades during pursuit than the other groups. Many of these saccades appeared to anticipate target motion. During a saccadic stepping task, schizophrenics made more maladaptive anticipatory saccades than the other groups. 50% of the schizophrenics had rates of maladaptive anticipation more than 2 SD above the mean of the controls. Saccadic latency and velocity did not differ among the groups, but schizophrenics often made hypometric visually guided saccades. During a step-gap saccadic task which involved learning to make adaptive anticipatory saccades, 6 schizophrenics failed to anticipate; only one control failed to learn. Schizophrenics also tended to make more variable anticipatory saccades (both amplitude and timing) and to anticipate sooner than controls. These results suggest that the most characteristic eye movement abnormality in schizophrenia involves excessive and maladaptive saccadic anticipation. Dopaminergic dysfunction in the basal ganglia thalamocortical oculomotor loop may disinhibit the superior colliculus lowering the threshold for anticipatory saccades.

475.18

SMOOTH PURSUIT EYE MOVEMENT DYSFUNCTIONS IN SCHIZOPHRENICS
A.Mackert* and M.Flechtner* (SPON: J.Kasper). Dept. Psychiatry, Free University of Berlin, 1000 Berlin 19 (West) F.R.G.

Eye tracking dysfunction (ETD) is believed to be a possible psychophysiologic correlate of schizophrenia. The question of whether ETD is a trait in functional psychosis or a motor side effect of neuroleptics bears on the theoretical significance of the phenomenon.

We analyzed smooth pursuit tracking in 47 acute inpatients fulfilling RDC-criteria for schizophrenia. No patient was treated with neuroleptics at the time of the examination. 15 patients had never received psychopharmacological treatment. 26 remitted patients were followed up after a mean duration of 11.5 weeks on neuroleptic therapy. Eye tracking was recorded with DC-EOG. The target was driven sinusoidally at a frequency of 0.4 Hz and an amplitude of 20° of visual arc.

The prevalence of ETD among schizophrenics was 45% compared to 12% of sex- and age-matched normal controls. There was no difference between previously neuroleptic treated and drug-naive schizophrenics. A high test/retest stability could be found between the first and second testing despite a significant reduction in the severity of psychotic symptoms after neuroleptic therapy. These findings suggest that the ETD is a trait characteristic of schizophrenia and independent of neuroleptic medication and clinical state.

475.19

PREDICTIVE AND VISUALLY-DRIVEN COMPONENTS OF SINUSOIDAL SMOOTH PURSUIT EYE MOVEMENTS.
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The aim of our study was to determine whether monkeys utilize a nonvisual predictive signal as a command for eye acceleration during smooth pursuit of sinusoidally-moving targets. In an earlier study (Soc. Neurosci. Abstr., 11:79, 1985) we used cross-correlation analysis to obtain the transfer functions relating eye acceleration to visual error signals. A computer model based on these transfer functions accurately simulated pursuit of targets whose velocity varied transiently and unpredictably. However, we have found that a model based on visual error signals alone cannot accurately simulate pursuit of sinusoidal target motion.

In the present study monkeys pursued targets moving sinusoidally over a range of frequencies and amplitudes. Cross-correlation analysis of eye acceleration vs. target phase revealed a "predictive" component of eye acceleration with a gain of approximately 0.7 in phase with target acceleration. Components related to visual errors were also obtained having approximately the same gains as those obtained for pursuit of unpredictable target motion; gains of these visual error components were independent of target phase. Total eye acceleration behaved as the linear summation of visual and predictive components. Inclusion of the predictive component in our computer model greatly improved the accuracy of its simulations. We conclude that the regularity of sinusoidal target motion allows monkeys to use predicted target acceleration as a feedforward command to improve pursuit performance.

(Supported by NIH Grant EY03878)

475.20

PRECISION SMOOTH PURSUIT EYE MOVEMENTS REQUIRE MOTOR LEARNING
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It has been suggested that the primate's repertoire of eye movements is largely innate. Improved performance that is frequently noted during training on oculomotor tasks is often attributed to cognitive factors (better understanding of the task or higher motivation) and not motor learning. We have evidence that the smooth pursuit system requires considerable practice to develop the motor skills needed to maintain the fovea on a moving target. Initially, the monkey was presented with a small spot of light which ramped away from the center with only vertical motion. Target direction and speed were randomized, and reinforcement was given if the animal met tight eye position criteria during pursuit. After five days of training, eye acceleration in the first 100msec of pursuit had almost doubled. At this point, horizontally ramping targets were first introduced. Over the first hour, acceleration in the first 100 msec of horizontal pursuit was at least 1.5 times slower than vertical, and inappropriate vertical eye movements were consistently made. Successive days of continued horizontal training progressively decreased these differences. Monkeys that have received months of training on smooth pursuit tasks show, on average, equal initial vertical and horizontal accelerations. Work is underway in a second animal to verify these findings. These results show that the smooth pursuit system requires experience for optimal performance even when motivation and task knowledge are high.

(Supported by a Rachael Atkinson Fellowship and EY06860)

476.1

PARALLEL COMPUTER MODEL OF THE *LIMULUS* LATERAL EYE. Ramkrishna Prakash*, Eduardo Solessio* and Robert B. Barlow, Jr., Institute for Sensory Research, Syracuse University, Syracuse, NY.

The retina of the *Limulus* eye is the largest neural network for which a quantitative model exists. In a pioneering study, Hartline, Ratliff, and their colleagues formulated a neural network model that describes the steady-state responses of single optic nerve fibers in terms of the properties of single neurons and the interactions among them. Subsequent studies extended the model to include essential nonlinearities and temporal properties of excitation and inhibition.

Parallel computers are ideal for modeling neural networks such as the *Limulus* retina. We have developed a time-dependent model of the retina on the Connection Machine (CM 2, 32,000 processor). The model uses 8,192 processors to represent a matrix of 64x128 retinal receptors. We simulate light transduction and adaptation of receptors with the Hodgkin-Fuortes model and the interactions among receptors with digital filters.

Connection Machine computations match to first approximation of the patterns of neural activity recorded in the laboratory in response to moving patterns of illumination. We will report how the model responds to changes in the spatio-temporal properties of stimulus patterns as well as to changes in the spatio-temporal parameters of the model itself. The latter are important because we now know that the properties of the retina are modulated from day to night by a circadian clock. Computations with "daytime" and "nighttime" network models will hopefully provide a better understanding of the neural basis of the animal's visual behavior.

Supported by NSF 8709059 and NIH grant EY-00667.

476.2

PARSIMONY OF NEURAL CONNECTIONS: COMBINATORIAL ADVANTAGE OF GLOBALLY SIZE SCALING INPUT PATTERNS. R. B. Glassman. Dept. Psychol., Lake Forest Col., Lake Forest, IL 60045.

A great theoretical puzzle is how astronomical numbers of neural connections in the brain categorize still larger numbers of input pattern variations. One way may involve size scale restrictions. Consider a hypothetical sheet of N cells. Another neural subsystem, restricted to receiving input from M of these units at a time, must be prepared to read any of C(N,M) input patterns. If the receiving subsystem is further restricted to scaled input patterns whose activated elements are multiples of $\sqrt{u}$ X $\sqrt{u}$ elements apart, it must read (N)(C((N/u),M)) patterns, which is usually a smaller number. This will be illustrated in a family of curves. (The leading multiplier (N) ensures that each of the C((N/u),M) patterns is read at every possible position. For simplicity, the element sheet is treated here as if it had no edge, or as if N were very large relative to M.)

The ratio [C(N,M)]/[(N)(C((N/u),M))] is the "combinatorial advantage" of size scaling. When both N and N/u are very large relative to M, the formula u^M/N gives the approximate combinatorial advantage.

One use of this line of analysis might be in considering size scaling and connectivity of the multiple cortical mappings of the retina.

476.3

INTERPOLATION BY PHOTORECEPTOR ARRAYS. J.H. van Hateren* (SPON: T. Poggio). Dept. of Biophysics, Westersingel 34, NL-9718 CM Groningen, The Netherlands.

Photoreceptor arrays can be used for highly specialized visual tasks. For instance, dorsal eyes of males of many insect species are specialized for tracking females seen against the sky (e.g. van Hateren et al., J.Comp.Physiol.A 164:297-308). This raises the question of how precise a photoreceptor array can determine the position of an object much smaller than the halfwidth of the photoreceptors' angular sensitivity. I analysed this in the 1-dimensional case for interpolation by two photoreceptors. I found that the object position x can be determined with an uncertainty $\Delta x = \sqrt{(r_1(x)\Delta r_2(x))^2 + (r_2(x)\Delta r_1(x))^2}/|r_2(x)r_1'(x) - r_1(x)r_2'(x)|$, where $r_1(x)$ and $r_2(x)$ are the angular sensitivities of the two photoreceptors, $\Delta r_1(x)$ and $\Delta r_2(x)$ their uncertainties, and the prime denotes a derivative to x. If a small object is seen against a bright background, it will cause only a small modulation in the total light flux a photoreceptor receives, and in good approximation $\Delta r(x)$ will be independent of x (mainly determined by variations in background photon flux and by transducer noise). This leads to $\Delta x = \Delta r\sqrt{r_1^2(x) + r_2^2(x)}/|r_2(x)r_1'(x) - r_1(x)r_2'(x)|$. In general, the uncertainty Δx in object position will depend on x, i.e. where the object happens to be. However, if $r_1(x) = \cos(ax)$ (for $-\pi/2a \le x \le \pi/2a$, $r_1(x) = 0$ elsewhere) and $r_2(x) = \cos(ax - \pi/2)$ (for $0 \le x \le \pi/a$, $r_2(x) = 0$ elsewhere), with a a scaling constant, Δx is independent of x if x lies between the photoreceptors' visual axes ($0 \le x \le \pi/2a$). These functions thus yield a position independent precision for determining object position, but lead to an undersampling by the photoreceptor array of about 1.5 times (see also Snyder et al., Science 231:499-501).

Supported by the Netherlands Organization for Scientific Research.

476.4

LEARNING RECEPTIVE FIELDS THAT ARE MATCHED TO AN IRREGULAR PHOTORECEPTOR LATTICE. L. T. Maloney* (SPON: R. M. Shapley). Department of Psychology and Center for Neural Science, New York University, New York, New York 10003.

A method is described that reorganizes receptive fields of a model visual system so as to compensate for irregularites in the photoreceptor lattice. It makes use of visual information in patterned visual scenes, can correct for optical distortions, and could supplement other chemical and electrical cues active in visual neural development.

The method requires that the visual system be able to compute transformations t that would compensate for eye movements *if* the receptive fields in the visual system were matched to the photoreceptor lattice. If the receptive fields and lattice are not matched, the method uses the transformations to generate an error signal by comparing visual input across eye movements. The error signal guides reorganization. The method requires no knowledge of the contents of a particular visual scene, no feedback, other than the error signal it itself computes, and could be implemented by many adaptive algorithms. Simulations indicate that the method is little affected by small errors in eye position information.

476.5

DISTRIBUTION AND MORPHOLOGY OF HUMAN CONES STAINED WITH ANTI-BLUE CONE OPSIN. C.A. Curcio, K.A. Allen, C. Lerea, J. Hurley, I. Klock, & A. Bunt-Milam. Depts. of Biol. Structure, Ophthalm., & Biochem., & HHMI, U. Washington, Seattle WA 98195

Primate cones maximally sensitive to short wavelength light (blue or B-cones) have been previously identified using indirect methods. We stained whole mounted human retinas obtained from immersion-fixed donor eyes, using an affinity purified antibody to a 19 amino acid peptide sequence at the N-terminus of B-cone opsin, standard PAP immunocytochemistry, and controls. Cones were counted where all outer segments could be traced to inner segments and were measured where cells were well aligned vertically.

In 4 retinas from 3 females, we find that **1)** foveal B-cones are sparse, irregularly spaced, and are missing in a zone (max. diam. 100 μm) adjacent to the site of peak cone density. **2)** The highest B-cone densities (>2000 cells/mm^2) are found in a ring at 100-300 μm eccentricities, with peak density 100 μm superior and inferior to the foveal center. These findings are consistent with psychophysical reports of maximum sensitivity to blue light at 1° eccentricity and suggest a radial asymmetry for B-cones that differs from the overall cone mosaic. **3)**1% of cones within 100 μm of the foveal center and 8-10% of cones at 3-4 mm eccentricity are B-cones. **4)** B-cone inner segments tend to be slightly larger than red/green cones near the junction with the outer segment and slightly smaller at a more vitread level. EM immuno-gold studies will determine whether these findings reflect differing size, shape, or vertical position of B-cones.

476.6

IMMUNOCYTOCHEMICAL IDENTIFICATION OF CONES IN THE RETINA OF NOCTURNAL AND DIURNAL PRIMATES K. C. Wikler and P. Rakic. Sect. Neuroanat., Yale Sch. Med. New Haven, CT

An antibody specific to cone photoreceptors was used to determine their morphology and distribution in the retina of four primate species that differ in their capacity for color vision. We found that the monoclonal antibody, CSA-1 (Johnson and Hageman, 1988), applied to retinal wholemounts of Old World macaques (*Maccaca mulatta* and *M. fascicularis*), labels cones exclusively with a preference for the red/green- and not the blue-sensitive outer segments. In the retina of the nocturnal New World owl monkey (*Aotus trivirgatus*), which lacks a central peak in cone density, immunocytochemistry reveals an orderly array of labelled cone outer segments across the retinal surface. Surprisingly, a nocturnal prosimian (*Galago garnetti*), which possesses poor color vision and was thought to have few if any cones, has a similar distribution of immunoreactive cells per unit area of retina. Subsequent examination of wholemounts using video-enhanced differential interference optics showed that immunolabelled photoreceptors in the galago have a wider outer segment and shorter myoid than unlabelled receptors, and thus should be considered as cones rather than rods. Additionally, CSA-1 labels only red/green-sensitive cones in both nocturnal primates. Although all four species had a similar packing density of CSA-1 reactive cones per unit area outside of central retina, the diameter of cones, the number of cones per degree of visual angle, and the cone to rod ratio were smaller in nocturnal species which may explain their poor color vision. Immunocytochemical markers help to gain insight into the development and evolution of photoreceptors subserving color vision in primates. Supported by EY 02593 (P.R.) and NS 0859 (K.C.W.)

476.7

SPATIAL DISTRIBUTION OF CHOLINE ACETYL-TRANSFERASE (ChAT) LABELED CELLS IN THE MACAQUE RETINA. R. W. Rodieck and D. W. Marshak. Department of Ophthalmology, University of Washington, Seattle WA 98195; Department of Neurobiology and Anatomy, University of Texas Medical School, Houston TX 77225.

Whole-mounted macaque retinas were used to determine the spatial density of ChAT immunoreactive cells. Previous work has shown that these cells correspond to the starburst amacrine cells, and that, in primates, almost all their somata lie in the ganglion-cell layer. The density profile has the overall shape of a steep-sided volcano, centered on the fovea. The density on the foveal slope is about 1200/mm^2, rises to about 1600/mm^2 at 1 mm eccentricity, drops rapidly to about 400/mm^2 at 4 mm, and then gradually decreases to about 100/mm^2 near the ora serrata. To a first approximation the distribution is radially symmetric; however, in common with other cell types of the primate retina, the density at a given eccentricity in the peripheral retina is higher in the nasal retina than in the temporal retina. The total number of ChAT-labeled cells in a retina is about 120,000.

Autocorrelation analysis (a generalized nearest-neighbor measure) showed that the somata in any local region form a quasiperiodic array. The overlap factor (spatial density x dendritic-field area), based upon these density measurements and dendritic-field sizes as determined by either Golgi impregnation or intracellular filling with horseradish peroxidase (HRP), is about 20 for all regions outside the fovea.••The antiserum was provided by Dr. Louis Hersh at NIH. Supported by NIH grants EY02523, EY01730, EY06472 and the Oregon Regional Primate Center.

476.8

DOG RETINAL GANGLION CELLS: MORPHOLOGICAL TYPES AND BREED DIFFERENCES IN TOPOGRAPHY. L. Peichl, Max-Planck-Institut f. Hirnforschung, Deutschordenstr. 46, 6000 Frankfurt 71, FRG

The morphological types of ganglion cells in the dog retina and their topographical distribution were studied with intracellular injection of Lucifer Yellow, and with reduced silver and Nissl staining. Ganglion cells with large somata had large alpha-type dendritic trees; most cells with medium-sized somata had small beta-type dendritic trees; cells with small somata had a variety of dendritic branching patterns. All resembled the types found in cat retina. Alpha and beta cells divided into inner and outer branching subtypes, presumably representing ON and OFF channels.

Ganglion cell topographies, quantified in Nissl-stained wholemounts, varied markedly between dogs. Maximal densities in the central area ranged from 7,500 to 14,000 cells/mm^2. The topography in many German shepherd dogs was similar to that seen in cat retina with a moderate horizontal streak. In contrast, many Beagles had a very pronounced horizontal streak of high ganglion cell density extending into nasal periphery. Intermediate densities and lengths of streaks were found in other breeds, and intra-breed variability was common.

Alpha cells comprised 4-12% of the ganglion cells depending on retinal location. However, in all breeds, alpha cells were lacking in a substantial region of temporal retina. Thus functional deficits of the alpha cell (presumed Y) pathway might be expected in that part of the visual field.

476.9

GANGLION CELLS IN THE HAMSTER RETINA. C. Métin* and D.O. Frost. Inst. des Neurosciences, Université P. & M. Curie, Paris, France and Dept. of Neurology, Massachusetts General Hospital, Boston, MA. 02114

In order to extend our anatomical and physiological findings on the plasticity of retinal projections, we have studied the distribution and the different morphological classes of ganglion cells (GCs) in whole, flat mounted retinae of normal adult hamsters. GCs were identified by backfilling with horseradish peroxidase (HRP), a criterion not used in previous studies of hamsters. A dried pellet of polyacrylamide gel containing 30-35% HRP (Griffin et al, Br. Res., 168:595, 1979) was inserted just ventral to the ventral lateral geniculate nucleus in a cut in the optic tract (OT). After 72h, the hamsters were perfused with PBS and their flattened retinae immersion fixed in 1.25% glutaraldehyde. Labeled GCs were revealed by processing the retinae with a modified Hanker-Yates method (Hanker et al, Histochem. J. 9:789, 1977; Adams, J. Histochem. Cytochem. 29:775, 1977). In retinae ipsilateral to the treated OT, labeled GCs were present inferotemporally in a peripheral crescent 1600-1800 µm wide. GC density was highest in the superior half of the crescent (300-400 GCs/mm^2). Contralaterally, labeled GCs were distributed throughout the entire retina but showed significant variations in density; density decreased from the center (2000-2200 GCs/mm^2) to the periphery (250-500 GCs/mm^2) and tended to be higher in the inferior hemiretina. A region of high density (2000 GCs/mm^2) was also observed in the inferotemporal retina, between the optic disc and the internal border of the temporal crescent. GC diameter varied from 6 to 26 µm. The distribution of soma diameters for GCs projecting in the ipsilateral OT was broad, with most GCs between 10 and 22 µm; the distribution peaked in the range of 16-19 µm and fell off slowly toward lower values and more rapidly toward higher values. The soma diameter distribution for contralaterally projecting GCs was unimodal with a peak at 10-12 µm and a tail elongated toward higher values; GCs in peripheral retinal regions tended to be larger than those more centrally.

The best filled GCs could be classified as belonging to one of the 3 types distinguished in the rat retina by Perry (Proc. Roy. Soc. B 204:363, 1979) according to their dendritic morphology and soma diameter. Variations in soma size with intraretinal position will be related to variations in GC density and morphological type. Immunocytochemical data on some GC classes will also be presented.

Supported by EY-03465 from NIH, 1-1060 from March of Dimes, 0212/87 from NATO, UA1199 from CNRS and La Fondation de France.

476.10

THE ORGANIZATION OF GANGLION CELL FASCICLES IN THE RETINAS OF PIGMENTED AND ALBINO RATS. W. L. Holcomb,* D. M. Murakami,* I. S. Westenberg[1] and C. A. Fuller. (SPON: I.H. Perline) Dept. of Animal Physiology, University of California, Davis, CA 95616; [1]Dept. of Psychology, Glendale College, Glendale, AZ 85302.

This study compared the organization of retinal fascicles in the pigmented rat with that of the albino rat. In order to control for the possibility of variation due to rat strain underlying the anatomic differences in fascicle organization, a specific strain was used (Westenberg Long-Evans) in which litter-mates contained both pigmented and albino rats.

The optic nerve head was injected with HRP. The eyes were placed in oxygenated ringers for 3-4 hours, briefly fixed, and the retinas were reacted for HRP. Peak isodensity lines of the fascicles in the pigmented rats were located near the presumed area centralis in the temporal retina. In contrast,the albino retinas exhibited isodensity lines with a more symmetrical pattern around the optic disc. The distribution of individual fascicle size was examined along the isodensity lines in different regions of the retina. The fascicles in pigmented rats were smallest near the regions of peak isodensity. The fascicle size became larger away from the region of isodensity specialization (presumed area centralis). A similiar organization of fascicle size was found in albino rats despite the lack of isodensity specialization.

476.11

PRESUMPTIVE CATECHOLAMINERGIC RETINAL GANGLION CELLS PROJECT TO THE VENTRAL LATERAL GENICULATE NUCLEUS IN THE PIGEON. K.T. Keyser*, L.R.G. Britto and H.J. Karten. Dept of Neurosciences, UCSD, La Jolla, CA 92093.

Recent studies have demonstrated heterogeneity in both putative transmitter content and central targets of specific populations of retinal ganglion cells (RGCs). Immunohistochemical surveys of possible transmitters used by RGCs revealed that approximately 5000 cells in the ganglion cell layer (GCL) of the pigeon retina exhibited tyrosine hydroxylase (TH) immunoreactivity and are thus presumptively catecholaminergic. These cells varied in size from 8-20 μm depending upon eccentricity. Following injections of tracer into the contralateral nucleus geniculatus lateralis, pars ventralis (GLv), 800-1500 of the TH-positive cells were also labeled with the tracer. The double labeled cells were 8-15 μm in diameter. In other experiments, some TH-positive cells in the GCL of the pigeon were labeled following injections of tracer into the optic tectum (OT) suggesting that axons of some of the TH-positive cells might project to both areas. Therefore, a green fluorescent tracer was injected into the OT and a red fluorescent tracer was injected into the GLv. TH-positive cells in the contralateral retinae were visualized with a blue fluorophore. In these experiments, 330 to 450 cells exhibited fluorescence characteristic of all three fluorophores. Thus, a subpopulation of small, biochemically distinct ganglion cells in the avian retina projects to the GLv. In addition, this study confirms suggestions from previous reports in both birds and mammals that some retinal ganglion cells that project to GLv also project to other targets. Supported by EY07845 (KTK), FAPESP and CNPq (Brazil, LRGB) and EY06890 (HJK).

476.12

THE NORMAL, DIRECT IPSILATERAL RETINO-TECTAL PROJECTION IS MIRROR-SYMMETRIC TO THE CONTRALATERAL PROJECTION IN RANA PIPENS. F. Scalia, E.L. Singman* (SPON: K. Fukada) Department of Anatomy and Cell Biology, SUNY-Health Science Center at Brooklyn, Brooklyn, N.Y. 11203.

It has been known since the introduction of the highly sensitive autoradiographic and horseradish peroxidase (HRP) axon tracing techniques that a small part of the retinal ganglion cell (RGC) projection in the normal frog enters the ipsilateral optic tectum. Although its size increase after optic nerve regeneration (Stelzner, 1981), this projection has been difficult to detect electrophysiologically (it is not the relayed isthmo-tectal projection that maps the binocular field homotopically onto the anterior region of the ipsilateral hemisphere), and earlier attempts by others to locate the RGC of origin of the normal projection were unsuccessful. In the course of observations on the retrograde transport of HRP after unilateral, intense injections into the tectal locus that maps the central nasal region of the contralateral retina, we have observed cellular localization in the ipsilateral retina. Although only a small number of RGC are labeled in the ipsilateral retina in our preparations, which are examined as flat-mounts, they are clustered in the correct retinal locus, i.e., the RGC labeled in the retinas from both eyes are centered in mirror-image locations in the central nasal retina. These observations strongly argue for uptake of HRP by terminals rather than by fibers crossing the tectum in passage. The small size of this synaptic projection may account for the difficulty in detecting it physiologically, although, since it is mirror-symmetric, it may be subjected to active suppression within the tectum. Supported by PHS grant NO. EY05284 to F.S.

476.13

CELLS OF DOGIEL AND GANGLION CELL DISPLACEMENT DURING OPTIC NERVE REGENERATION, E.L. Singman* and F. Scalia. Department of Anatomy and Cell Biology, SUNY-Health Science Center at Brooklyn, Brooklyn, N.Y. 11203.

Cells of Dogiel (DC) are retinal ganglion cells (RGC) whose perikarya are normally located among the amacrine cells in the inner nuclear layer in the retina of many species. Their dendritic trees are inverted in the sense of extending vitread into the inner plexiform layer (IPL). There is evidence (Montgomery et al., '81) that some of these cells project to the basal optic nucleus in the frog. In a previous study (Scalia et al., '85), using a flat-mounting technique, we observed massive RGC death in R. pipiens after periods of optic nerve regeneration and reported for the first time that large numbers of RGC with normally oriented or tangentially spreading dendritic trees were displaced bodily into the IPL in such specimens. We subsequently injected horseradish peroxidase (HRP) into the tectum bilaterally at loci which map the middle region of the nasal retina in 33 frogs surviving unilateral optic nerve transection for periods of 3-89 wks. Retrograde transport deposited HRP in RGC in the main ganglion cell layer in both eyes, but not in DC in either eye. Therefore, DC do not project to the tectum, and do not come to project there aberrantly during regeneration. In the retinas substaining optic nerve regeneration, the RGC newly displaced into the IPL, as well as the non-displaced RGC, were labelled with HRP in significant numbers. In a typical retina sectioned after completing observations on it as a flat-mount, the thickness of the IPL measured at 219 locations was 14.3 +/- 0.39 um in the affected eyes, and the mean outward dislocation of the displaced cells was 49.3 +/- 1.7% of that distance (N=219). The non-displaced RGC formed a monolayer, as in the normal retina. Supported by PHS grant EY05284 to F.S.

476.14

MORPHOLOGY AND PROJECTION SITES OF FROG RETINA GANGLION CELLS (RANA ESCULENTA). U. Grüsser-Cornehls*, J. Pipke* and B.G. Grover* (SPON: ENA). Dept. of Physiology, Freie Universität Berlin, Arnimallee 22, D-1000 Berlin 33, FRG.

Morphological investigations were undertaken in the retina of the frog (Rana esculenta) using either the Golgi or the BHRP (B-subunit of cholera toxin conjugated to horseradish peroxidase) technique.

In our sample of 48 cells encountered with the Golgi technique (sagittal sections) we could distinguish 11 main types of retinal ganglion cells. In order to determine their projection sites, BHRP was injected either into the tectum or the diencephalon. Frog retinas were flat-mounted and processed with DAB. By using then a careful "depth-differentiating" drawing method, dendritic abors could be reconstructed and cells correlated to those found with the Golgi technique. 200 cell drawings were analysed. The 11 morphological cell types (M1-M11) found with the Golgi technique could be confirmed. From these 11 types, 5 could be found after tectum and diencephalon injection, 4 only after tectum injection and one type, called M11, only after diencephalon injection. The dendritic tree of the latter type only spread within the fourth sublayer of the inner plexiform layer (IPL) - the most vitreal sublayer. The dendrites dived no deeper than 10 μ into the IPL and encompassed an area of $\approx$ 160 μ diameter radiating in all directions. The greater soma diameter varied between 14.6 and 16.1 μ. These cell types are probably the "blue-sensitive" on-units of the N. Bellonci (Muntz W. R. A.: J. Neurophysiol. 25: 699, 1962).

Supported in part by the Deutsche Forschungsgemeinschaft (Gr 276).

476.15

DO ALL RETINAL GANGLION CELLS CONTAIN N-ACETYLASPARTYLGLUTAMATE? S.B. Tieman and K.R. Fry. Neurobiology Research Ctr, SUNY, Albany NY 12222 and Ctr for Biotechnology, Baylor College of Medicine, The Woodlands TX 77381.

We have previously shown that many retinal ganglion cells (RGC) in the cat are immunoreactive for N-acetylaspartylglutamate (NAAG), a possible neurotransmitter. To determine whether all RGC contain NAAG, and whether all NAAG-positive cells in the ganglion cell layer are ganglion cells, we have attempted to double-label these cells with antisera to NAAG (provided by J.H. Neale of Georgetown Univ.) and with AB5, a monoclonal antibody specific for RGC. Three cats were deeply anesthetized and perfused with a mixture of 4% paraformaldehyde and 4% carbodiimide. Large pieces of intact retina were repeatedly frozen and thawed, then incubated for 10 days at 4°C in a mixture of the primary antibodies (anti-NAAG at 1:1500; AB5 (purified ascites fluid) at 1:10,000), followed by fluorescein-labeled donkey anti-rabbit and rhodamine-labeled goat anti-mouse (each at 1:100) for 2 days. The great majority of labeled cells contained both labels. However, some small cells were labeled for NAAG but not for the AB5 antigen. These were more common in peripheral than central retina, and we assume that they are displaced amacrine cells. In addition, a very few cells were AB5 positive but did not label for NAAG. We conclude that some displaced amacrine cells and all but a few RGC contain NAAG. (Supported by NSF grant BNS-8811039 to SBT and PHS grant EY06469 to KRF.)

476.16

IMMUNOHISTOCHEMICAL LOCALIZATION OF GABA-A RECEPTORS IN THE RETINA OF THE CAT. T.E.Hughes, U.Grünert*, H.J. Karten. Dept. of Neurosci., UCSD, La Jolla, CA, *Max-Planck-Inst. für Hirnforschung, Frankfurt.

GABA, an inhibitory neurotransmitter, is present in approximately 25-30% of all amacrine cells in the retina of the cat (Wässle & Chun, J. Comp.Neurol., 279:43-54). To better identify the synaptic targets of these cells, we undertook an immunohistochemical study of the GABA-A receptors. We used a monoclonal antibody (62-3G1), kindly donated by Dr. Angel de Blas, which recognizes a 57 KD subunit of the receptor (Victorica et al., J. Neurosci. 8:615-622).

The antibody labels somata in the inner nuclear (INL) and ganglion cell (GCL) layers and a dense plexus of processes in the inner plexiform layer (IPL). The labeled cells in the INL appear to be amacrine cells. They have small somata (5-7µm in diameter) located next to the IPL. They range in density from 800/mm² in the peripheral retina to 2500/mm² in the central regions. In the GCL, the antibody labels small somata, quite likely "displaced" amacrine cells or ganglion cells (gamma cells), and medium-sized ganglion cells (beta cells). The large alpha cells are not clearly labeled. In the IPL the labeled processes spread throughout the layer, but there is a strong concentration in the vitreal third of the layer, suggesting that GABA-A receptors are strongly involved in the scotopic pathway through the IPL. This pattern is exactly the opposite of the glycine receptor (Jäger & Wässle, Neurosci. Lett., 75:147-151).

476.17

DOPAMINERGIC AND SEROTONINERGIC NEURONS IN THE XENOPUS RETINA. M. Schuette* and P. Witkovsky. Dept. Ophthalmology, NYU Medical Center, New York, NY 10016.

Anti-tyrosine hydroxylase stains a population of cells in the Xenopus retina. The soma (12-15 um diameter) is located in the amacrine cell layer. The cells are unevenly distributed with the highest density of ca. 200 cells/mm2 in the caudal lateral part of the retina whereas the frontal medial part contains only a few scattered cells. About 2% of the cells were observed to have processes ascending within the inner nuclear layer. Within the inner plexiform layer (IPL), thick dendrites form a sparse network in the proximal portion, whereas in the distal portion a dense network of fine processes was seen that forms rings around perikarya. Preliminary data suggest that some of these perikarya exhibit glycine-like immunoreactivity (as noted by Voigt & Waessle in the cat retina).

Serotonin-like immunoreactivity stains two classes of amacrine cell, the large soma amacrine (L-ser, soma size 12- 20 um) has several primary dendrites, the small soma amacrine (S-ser, 8-10 um) exhibits only one major process. Processes of both classes ramify diffusely in all sublayers of the IPL. TOH and serotonin-positive cells have a similar distribution, the ratio of the TOH, L-ser and S-ser cells is ca. 2:1:5. Additionally two classes of bipolar cells show serotonin-like immuno-reactivity. Supported by EY 03570 to P.W.

476.18

EVIDENCE FOR ADDITIONAL CHOLINERGIC LAMINAE IN THE INNER NUCLEAR LAYER OF THE GOLDFISH RETINA. A.D. Springer, B. Wilson*, and K. Morel*. Dept. Of Cell Biology and Anatomy, New York Medical College, Valhalla, NY 10595.

An antibody to choline acetyltransferase (AB8) was applied to flatmounted retinae having a mean area of 21 mm². The retinae were embedded in either paraffin or plastic and sectioned, or were examined as flatmounts. A bimodal distribution of cholinergic cell areas was present in both the inner nuclear (INL) and ganglion cell layers (GCL). The density of small cells (15 μm^2) in the INL was 2,227 mm² and that of large cells (50 μm^2) was 739 mm². Small cells (20 μm^2) in the GCL had a density of 1,090 mm², and large cells (60 μm^2) in the GCL were rare (4 mm²). Nasal and temporal retina had the same cell density distributions. As retinal area increased by a factor of 2.3, cell density decreased by 55%, and cell area increased by 25%.

Two major cholinergic bands were observed in the innerplexiform layer. As described by others, one was at 20% and the other was at 64% of the distance between the INL and GCL. Densitometry was used to analyze the laminar distribution of the antibody. The lamina closest to the INL comprised 2 bands, one at 14% and the other at 28% of the INL-GCL distance. In some, but not all, material the second major lamina (near the GCL) was also resolved into 2 bands. One band was at 60% and the other was at 66% of the distance between the INL and GCL.

476.19

THE DEVELOPMENT OF PUTATIVE GABAERGIC AND DOPAMINERGIC NEURONS IN THE RETINA OF THE ZEBRAFISH, BRACHYDANIO RERIO. C. Fulwiler, N. Mayer Benegas* & J.E. Dowling. Program in Neuroscience, Harvard Medical School; The Biological Laboratories, Harvard University, Cambridge, MA 02138.

The zebrafish is a rapidly-developing teleost which breeds easily in the laboratory and for which genetic methods exist. These features can be combined with our knowledge of the teleost retina to study retinal development and function. As the basis for future genetic studies, we are studying the normal development of the retina in this animal. Here we use immunohistochemistry to compare the development of the inhibitory transmitter GABA, and the neuromodulator dopamine, using antibodies against their synthetic enzymes glutamic acid decarboxylase (GAD) and tyrosine hydroxylase (TOH), respectively.

In the adult, staining is similar to that seen in other teleosts. GAD-immunoreactivity (GAD-IR) is present in amacrine and horizontal cells, the outer plexiform layer (OPL) and two bands in the IPL. TOH-IR is confined to interplexiform cells and their processes in both plexiform layers.

During development, both GAD-IR and TOH-IR are barely detectable in the retina at 2d, although staining elsewhere in the brain is intense. At this time, the IPL is just becoming apparent. By 3d, the time of hatching, retinal staining is distinct and adult-like in distribution. Two features distinguish the development of the two systems. First, TOH-IR is much slower to develop in intensity than GAD-IR. By 15d when GAD-IR is adult-like in intensity, TOH-IR lags far behind. Second, GAD-IR is seen in the optic nerve between 3 and 5d, but disappears by 9d and is not seen in the adult.

476.20

STATISTICAL ANALYSIS OF SYNAPTIC MICROCIRCUITRY OF A SERIALLY RECONSTRUCTED AMACRINE CELL WITH NPY-LIKE IMMUNOREACTIVITY IN THE TURTLE RETINA. W.D. Eldred and T. Isayama*. Department of Biology, Boston University, Boston, MA 02215.

In our previous studies in the turtle retina (Isayama and Eldred, 1988; Isayama et al., 1988), we classified three different types of amacrine cells with neuropeptide Y-like immunoreactivity (NPY-LI). In order to examine the synaptic microcircuitry of the type A amacrine cells with NPY-LI in more detail, a well-labeled example was serially sectioned and reconstructed. All of the synaptic contacts associated with this cell were located and characterized. The synapses were quantified in terms of the ratio of synaptic input to output, the synaptic distribution as a function of distance from the cell soma, and the synaptic distribution within the three primary levels of arborization in the inner plexiform layer. Five types of synaptic contacts (bipolar and amacrine cell input; output to amacrine, ganglion, and bipolar cells) were termed "target synaptic types". Statistical analyses were conducted on the number and types of synapses surrounding each of the different target synaptic types. Our results indicated that the numbers of synaptic contacts associated with each of these target synaptic types were not significantly different. However, there were significant differences in the types of synapses surrounding the target synapses, i.e., their corresponding microcircuitry. For instance, the microcircuitry associated with bipolar inputs was significantly different from any of the outputs; and the microcircuitries surrounding outputs to amacrine versus ganglion cells were different. It is possible that such differences in synaptic microcircuitry may play an important role in defining the anatomical basis of visual processing. This research supported by EY04785 to WDE.

476.21

Phenylethanelamine N-methyltransferase-containing neurons in the small ear pig retina

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The localization of epinephrine-containing neurons in the small ear pig retina has been detected by immunohistochemical localization of the epinephrine-synthesiging enzyme phenylethanelamine N-methyltransferase (PNMT). The PNMT-positive cells are observed in the outer tier of cells in the inner nuclear layer (INL) and their processes are observed as punctute structure in the outer plexiform layer (OPL). The cell soma size is around 10-15 um. These adrenergic neurons are suggested to be amacrine cells or horizontal cells. The pattern of distribution has been further confirmed by electron microscope (EM). This results are similar to PNMT-positive cells in ferret retina (Keyser et al 1987, 7(12):3996-4004), but difference with PNMT-positive cells in rat retina in which cell bodies are found in the INL and ganglion cell layer (GL) and their porcesses are found in the outer and inner strata of IPL (Hadjiconstantinou et al, 1984, Neuroscience 13:547-551 and D. Park et al 1986, 6(4):1108-1113)

Supported by NSC, Taipei, Taiwan, R.O.C., grant NSC-780412-B001-03.

476.22

ROD BIPOLAR CELLS SHOW PROTEIN KINASE C-LIKE IMMUNOREACTIVITY IN THE CAT AND OTHER MAMMALIAN RETINAE. U. Grünert, U. Greferath* and H. Wässle. Max-Planck-Institut für Hirnforschung, Deutschordenstr. 46, D-6000 Frankfurt, F.R.G.

Rod bipolar cells are a crucial link in the scotopic pathway to retinal ganglion cells (e.g., Kolb and Nelson, Vision Res. 23:301-312, 1983; Freed et al., J. Comp.Neurol. 266:445-455, 1987). Recently, Negishi et al. (Neurosci. L. 94:247-252, 1988) reported that rod bipolar cells in many vertebrate retinae can be labelled with an antiserum directed against protein kinase C (PKC). Using this antiserum Karschin and Wässle (J. Neurophysiol. subm.) identified rod bipolar cells in a dissociated rat retina.

We have now stained rod bipolar cells in cryostat sections of the retinae of cat, rat and macaque monkey. Staining was most intense in the outer plexiform layer where the rod spherules synapse with the rod bipolar cells. The cytoplasm of the cell bodies located in the upper inner plexiform layer as well as the axons and the axon terminals of the rod bipolar cells were also intensely labelled. By electron microscopy of cat retina labelled dendrites were always the central elements of the rod spherule synaptic invagination providing further evidence that the labelled cells are indeed rod bipolar cells. Thus the antiserum against PKC is a good means to distinguish rod from cone bipolar cells and can be used as a tool for quantitative analysis of these cell types.

476.23

IMMUNOCYTOCHEMICAL DISTINCTION BETWEEN INNER AND OUTER HORIZONTAL CELLS IN THE LAMPREY RETINA. K. Rubinson, S. Yazulla and K. M. Studholme*. Dept. of Physiol. Biophys., New York Univ. Med. Ctr., New York, NY 10016 and Dept. of Neurobiology and Behavior, SUNY, Stony Brook, NY 11794.

During the development of the retina in the larval sea lamprey, Petromyzon marinus, horizontal cells (HCs) are morphologically distinguishable years before the differentiation of other synaptically-related cell types (Rubinson and Cain, in press) and years before the expression of visual responses (Rubinson, et. al., 1977). Two tiers (OHCs and IHCs) are apparent in the adult but, in the larva, HCs are in a single layer.

Immunocytochemical studies were undertaken to see if OHCs and IHCs express unique patterns of immunoreactivity (IR). Retinal sections from transformed, landlocked sea lamprey were reacted to GABA or glutamate anti-sera and processed by an indirect PAP procedure. OHCs were GABA-positive as were their axons which, in oblique sections, appeared as a felt-work between OHCs and IHCs. IHCs were distinctly glutamate-positive, as were many small terminal processes in the OPL. Each tier appeared as an unbroken palisade of cell bodies and there was no evidence of any displaced cells of either immunoreactivity. GABA-IR in HCs is expressed in non-mammalian retinas and as a transient developmental feature in mammals. The finding of glutamate-IR in HCs appears to be novel. This distinction in IR between OHCs and IHCs in lamprey is significant for analyses of HC lineage and phenotypic expression. (Supported by NIH RO1 EYO-1682 to SY)

476.24

CALRETININ AND OTHER CALCIUM-BINDING PROTEINS IN RETINA. J.H. Rogers* (SPON: M. Hanley). Physiological Laboratory, University of Cambridge, Downing St., Cambridge CB2 3EG, U.K.

Calretinin and calbindin-D28 are two calcium-binding proteins with 60% homology, which are abundant in separate sets of neurons in chick brain and retina [Rogers, J.H., *J.Cell Biol.* 105, 1343 (1987), and *Neuroscience*, in press]. Their distribution has been investigated by immunohistochemistry in retinae of chick, cat, rat, and salamander (larval *Ambystoma tigrinum*).

Sections were incubated with rat antiserum against a ß-galactosidase-calretinin fusion protein, and with rabbit antiserum against calbindin (gift of Dr. E. Lawson), or with rabbit antiserum against another calcium-binding protein, parvalbumin (gift of Dr. C. Heizmann). Two-colour immunofluorescence was used.

In all species except rat, there were some cones positive for calbindin, and some bipolar cells positive for calbindin or calretinin. Rods were always negative. In all species, horizontal cells contained large amounts of one or other calcium-binding protein, and of all three in the cat. In all species, there were many amacrine cells and some ganglion cells positive for one or more of these proteins. In the cat, these included a class of ganglion cells (or displaced amacrine cells), which appear to be highly asymmetric and which stain strongly for calretinin.

This work is supported by the Wellcome Trust.

477.1

THE MATURATION OF RETINOGENICULATE TERMINALS AND ALTERATIONS AFTER 'LATE' UNILATERAL EYE ENUCLEATIONS IN SYRIAN HAMSTERS. S. Jhaveri* ,R.S. Erzurumlu, and G.E. Schneider, M.I.T., Cambridge, MA, 02139.

Three types of retinogeniculate terminals (R1, R2 and R3) have been identified in the adult hamster (Erzurumlu et al., Brain Res., 461:175-181, 1988), each having a differential distribution within the LGBd: Large R1 terminals are located medially, small, clustered R2 terminals form an outer shell lateral and caudal to the R1's and R3 terminals are ubiquitous.

We have studied the maturation of R1 and R2 terminals by applying HRP crystals to optic tract axons below LGBv and visualizing the labeled axons and their terminals with HRP-DAB reaction product. Neonatal hamsters on postnatal days 1, 9, 14, and 21 were used in this study. On PND1 and on PND9 (after segregation of ipsi- and contralaterally projecting axons in LGBd), very few terminals have a definitive, adult-like morphology. At the time of eye opening (PND14), immature R1-type terminals can be differentiated and clustersof preterminal and terminal axons are present laterally where R2 terminals will develop. However, it is not until PND21 that morphological features typical of mature R1 and R2 terminals can be delineated. Unilateral eye enucleation results in altered distributions of retinal terminals even when performed up to PND14, albeit the extent of the changes decreases with increasing postnatal age.

Thus retinogeniculate terminals achieve stable, adult-like morphology and distribution several days after the eyes have opened and visually evoked activity has commenced.

Supported by NIH grants EY05504, EY00126 and EY02621. Conducted in compliance with NIH Publ. No. 85-23, 1985.

477.2

RETINOGENICULATE FIBERS ARE ESSENTIAL FOR THE PROPER TIMING OF DENDRITIC APPENDAGE ELIMINATION IN THE DORSAL LATERAL GENICULATE NUCLEUS. J. Keith Sutton and Judy K. Brunso-Bechtold. Program in Cell Biology and Neuroscience, Department of Anatomy, Bowman Gray School of Medicine, Wake Forest University, Winston-Salem, NC 27103

In our ongoing study of the development of neuronal morphology in the dorsal lateral geniculate nucleus (dLGN), we have used a Golgi-Hortega method to compare dendritic development in the dLGN of normal and enucleated ferrets. Following bilateral enucleation at birth, normal neuronal classes develop; however, the timing of filiform appendage development is altered in the absence of retinal input. Normal and bilaterally enucleated ferrets were perfused at ages from birth to maturity. In both groups, filiform dendritic appendages on large, stellate, class 1 neurons appear at the end of the first postnatal month, about the time of eye opening in normal animals. In normal ferrets, all appendages increase in number until P56, before undergoing a significant reduction by P90. In normal adults, most appendages have disappeared, especially on proximal dendritic segments. In bilateral enucleates, there is also an exuberant production of dendritic appendages before a decline to normal levels by adulthood, but this decline is delayed. Thus, retinogeniculate fibers are not necessary for the development of dLGN cell classes, but are essential for the proper timing of neuronal maturation (EY05028).

477.3

NEUROGENESIS OF THE GENICULATE NUCLEUS IN THE FERRET. **Jean D. Peduzzi. Dept. of Physiol. Optics, Sch. of Opt., Univ. of Alabama at Birmingham, Birmingham, AL 35294.**

The dorsal lateral geniculate nucleus (LGN) of the ferret affords an opportunity to compare the development of on-center and off-center channels in the brain. Cytoarchitectonic studies of the ferret LGN (Sanderson, 1974; Linden et al., 1981) revealed sublamination of laminae A and A1 into inner and outer leaflets. The outer leaflets of the A laminae were found in a physiological study (Stryker and Zahs, 1983) to contain off-center cells while the inner leaflets contain on-center cells.

In this study, a single pulse of ^{3}H-thymidine was injected into the placenta of ferret fetuses at various developmental ages. After sacrificing these animals as adults, alternate sections were processed with standard autoradiographic techniques and with GABA antisera (Incstar) using immunohistochemical methods. Also, in some of these animals, cytochrome oxidase or AChE activity was localized histochemically to confirm the boundaries of the sublaminae (Kageyama & Wong-Riley, 1984; Henderson, 1987). The birthdates of neurons in both A laminae of the LGN and the medial interlaminar and perigeniculate nuclei were determined. In addition, GABA-immunoreactive neurons in the outer and inner leaflets of the A laminae were compared in terms of birthdate, density and soma size.

Results of this study indicate that neurogenesis of the ferret LGN begins on or shortly before embryonic day 20 and continues to embryonic day 30. (EY01338 and EY03039)

477.4

CHANGES IN THE DISTRIBUTION OF ANTI-FIBRONECTIN IMMUNOREACTIVITY IN THE DORSAL LATERAL GENICULATE NUCLEUS DURING DEVELOPMENT. Judy K. Brunso-Bechtold and Darrell Agee*, Program in Cell Biology and Neuroscience, Department of Anatomy, Bowman Gray School of Medicine, Wake Forest University, Winston-Salem, NC 27103.

Cell layers in the dorsal lateral geniculate nucleus (dLGN) segregate from a relatively homogeneous distribution shortly after segregation of the ipsilateral and contralateral retinogeniculate fibers but before eye opening. During this time synaptogenesis in the dLGN is ongoing. In the present study, we determined the distribution of fibronectin (FN) in the dLGN while these developmental events were taking place. At P0, anti-FN immunoreactivity is evident in the optic tract as well as medial to the dLGN. By P4, there is less label in the optic tract, although dense label in the lateral portion of the C-layers and at the dLGN-perigeniculate border is evident. During the early stages of cell layer segregation, P8 and P14, there is increasing evidence of immunoreactivity within the dLGN. By P16, this label clearly corresponds to the laminar border between layers A and A1 and the perimeter of perigeniculate, but little immunoreactivity remains in the optic tract or C-layers. Anti-FN immunoreactivity continues to be present between layers at P24 and P31. By P44, only a band of label in perigeniculate can be seen and in the adult, no pattern of anti-FN immunoreactivity is apparent (EY05028).

477.5

TOPOGRAPHY AND SPECIFICITY OF VISUAL THALAMOCORTICAL PROJECTIONS IN THE FETAL RHESUS MONKEY. B. Lia, C.J. Snider*, and L.M. Chalupa Dept. Psychology & Calif. Primate Res. Ctr., Univ. California, Davis, CA 95616.

We have examined developing thalamocortical projections in the visual system of the fetal rhesus monkey (*Macaca mulatta*). Punctate injections of red or green fluorescent latex beads, limited to the cortical plate and subjacent subplate, were made on either side of the V1/V2 border. Lamination had progressed across only the dorsal half of the lateral geniculate nucleus (LGN) at E95 (gestation: 165 days), whereas by E111 the LGN appeared fully laminated. As in the adult, injections in V1 labeled cells densely in the parvo- and magnocellular laminae, whereas injections in V2 labeled cells sparsely in the S layers and interlaminar zones. Labeled cells formed topographically ordered columns within the LGN. At E95, shortly after geniculocortical fibers invade the cortical plate (Rakic '79), a small number of ectopic cells were found outside of the labeled-cell columns, and these cells were scattered within the more ventral (less-mature) portion of the LGN. By E111 these rare ectopic projections were eliminated. Labeling in the pulvinar also exhibited topographic order and specificity similar to the adult. At least two projection zones were apparent, the one more weakly labeled from V1. These findings indicate that in the rhesus monkey regressive events play only a minor role in the establishment of the specificity and topography of thalamocortical projections to visual cortex.

(Supported by RR00169 from NIH)

477.6

NEURAL CONNECTIONS IN LATERAL GENICULATE NUCLEUS-VISUAL CORTEX COCULTURES. N. Yamamoto*, T. Kurotani*, K. Yamada*, & K. Toyama (SPON: Y. ODA). Dept. Physiol., Kyoto Pref. Univ. Med., Kyoto 602, Japan.

Neural connectivity was studied in coculture preparations (2-3 weeks in vitro) of rat fetal lateral geniculate nucleus (LGN) and newborn visual cortex (VC). Morphological studies using Nissl staining and retrograde labeling with HRP or a fluorescent dye (DiI) demonstrated that the VC explant retained almost normal laminar and columnar organization as well as neuronal morphology such as pyramidal or stellate cells and that the LGN explant contained multipolar and bipolar cells. Furthermore, the antero- and retrograde labeling indicated that LGN axons with characteristic arbors terminated in the granular layer of the VC and that the infragranular layer cells projected to the LGN explant. Electrophysiological studies of extra- and intracellular responses confirmed that afferent and efferent connections were established between the LGN and VC explants with normal laminar specificity. These findings suggest the existence of intrinsic mechanisms controlling the development of appropriate neural connectivity in LGN and VC.

477.7

IPSILATERAL VS. CONTRALATERAL FLASH EVOKED RESPONSES IN ENUCLEATED RAT PUPS. S.K. Itaya and S. Molotchnikoff. Dept. of Biomed. Sci., Univ. So. Ala., Mobile, AL 36688, and Dépt. des Sci. Biol., Univ. de Montréal, Montréal, Que.

When monocular enucleation in rats on the day of birth (P0) results in an expanded ipsilateral retinotectal projection from the remaining eye in adults, it is believed to be due to the survival of ipsilaterally projecting retinal ganglion cells which would normally die. If enucleation removes an inhibitor to development of the ipsilateral pathway, then flash evoked responses should appear bilaterally in the colliculi of enucleated neonates. While contralateral responses were first recorded on P12 as in normals, no ipsilateral response could be found up to P15, the oldest age studied thus far. Flash evoked potentials were recorded from the ipsilateral colliculus in a normal adult, demonstrating the sensitivity of the apparatus. The results suggest that after P0 enucleation ipsilateral pathway development lags behind contralateral development. The findings correlate with additional studies where P0 enucleation induces transsynaptic transport of tracer in the ipsilateral pathway, but 7 days after contralateral labeling.

477.8

BEHAVIOURAL CONSEQUENCES OF EARLY MONOCULAR ENUCLEATION IN THE NORTHERN NATIVE CAT (DASYURUS HALLUCATUS). C. Ellard and J. Nelson*. Department of Psychology, Mount Allison University, Sackville, New Brunswick and Department of Zoology, Monash University, Clayton, Victoria, Australia

Although the anatomical reorganization produced by monocular enucleation has been the subject of intensive investigation, there is much less information about the behavioural consequences of these early lesions. Pouch-young of the Northern native cat, a small carnivorous marsupial, received monocular enucleations at ages ranging from 13 to 32 days after migration to the pouch. Animals were tested as adults in a visual perimeter. Orienting head and body movements to food stimuli were videotaped and single-fram analysis was used to characterize the movements. Normal controls obtained food by means of a series of discrete head and body movements followed by a reach with the forepaws. The number of such movements depended on both the distance and eccentricity of the target. Monocular enucleates obtained the food using significantly fewer and larger head and body movements. On most trials, these animals employed one slow, continuous movement lasting from trial onset to the end of the reach, regardless of the location of the target. One explanation for these findings is that the expansion of the ipsilateral retinocollicular projection produced changes in the brainstem circuitry subserving orienting movements.

477.9

DEVELOPMENT OF RECEPTIVE FIELD PROPERTIES IN THE NUCLEUS TRACTUS OPTICI OF THE CAT

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Adult cats have a symmetric monocular horizontal optokinetic reflex (OKR), i.e. the eyes follow both stimulus directions with about equal gain (eye velocity/stimulus velocity). A prerequisite for this response is the presence of binocular neurons in the nucleus tractus optici (NTO). About 80% of the neurons can be activated by either eye in adult animals. Cortical lesions and early visual deprivation as well as strabismus abolish the NTO-binocularity and the symmetry of OKR. NTO-neurons come under the sole control of the contralateral eye and OKN through one eye can only be elicited with temporo-nasal stimulus movement in the visual field.

Kittens show an asymmetric OKN up to age of about 3-4 months. Therefore, we recorded NTO-neurons in 6 kittens aged 27-47 days to investigate the ocular dominance distribution in this structure. Also in these animals 72% of the neurons could be activated by either eye and all were direction-selective for ipsiversive movement. There was, however, a significant difference in that, in kittens only 6% of the neurons had equally strong influence from both eyes compared to 35% in adult animals. Thus, at the onset of the sensitive period both eyes are connected to the NTO-neurons but the ipsilateral eye's influence is much weaker. Balanced binocularity develops rather late during the sensitive period (probably only after three months). This explains why OKN is asymmetric in kittens. Visual deprivation or strabismus must even disrupt the feeble early influence of the ipsilateral eye on NTO-neurons.

477.10

DEVELOPMENT AND PLASTICITY OF THE SEROTONERGIC PROJECTION TO THE HAMSTER'S SUPERIOR COLLICULUS. B.A. Figley, R.D. Mooney, C. Bennett-Clarke, N.L. Chiaia and R.W. Rhoades (SPON: M. Foy). Dept. of Anatomy, Medical College of Ohio, Toledo, OH 43699.

The hamster's superior colliculus (SC) receives a dense serotonergic projection from the nucleus raphe dorsalis. In this experiment, an antiserum (Inc Star) directed against serotonin (5-HT) was used to describe the development of serotonergic afferents to the hamster's superior colliculus (SC) and to determine whether enucleation, at birth or in adulthood, altered the organization of this projection. Serotonin positive fibers were present in all SC laminae by the day of birth. This projection consisted of two networks of fibers, one that was radially oriented and a second that generally paralleled the SC surface. Fibers in both of these networks did not branch extensively, but gave rise to numerous varicosities along their respective courses. By the third postnatal day (P-3), the density of the tangentially oriented fibers just below the pial surface had increased substantially. By P-7, the projection to all laminae had increased in density and it was no longer possible to discern distinct radially and tangentially oriented fiber networks. By day 14, the 5-HT innervation pattern of the SC appeared adult-like. There was a dense meshwork of fibers in the superficial and intermediate gray layers and a somewhat lower density of 5-HT-positive axons in all of the other laminae, especially the stratum opticum. Removal of one eye on either the day of birth or in adulthood resulted in a marked increase in the density of 5-HT-positive fibers in the superficial gray layer of the SC ipsilateral to the remaining eye. After neonatal enucleation, the difference between the two sides was apparent by P-14. It could also be detected within two weeks after enucleation in adulthood.

Supported by EY 04170, EY 08015, BNS 85 00142, and funds from the State of Ohio Research Challenge.

477.11

PROTEIN COMPONENTS OF THE RETINOTECTAL ACTIVITY DEPENDENT MAPPING MECHANISM. P. Steen* and M. Constantine-Paton. Biology Department, Yale University, New Haven, CT 06511.

The NMDA receptor appears to be a component of the activity dependent mechanism which organizes the retinotopic map within the frog tectum. Chronic treatment with the NMDA antagonist, APV, disorganizes the retinotopic map (Cline and Constantine-Paton, 1988, Neurosci. Abstr. 14:674). In addition APV causes desegregation of eye-specific stripes in 3-eyed animals and treatment with NMDA itself sharpens the stripes (Cline, et. al, 1987, PNAS 84:4243). These results suggest that NMDA receptor activation triggers a cascade of events leading to the ordering of the retinotopic map through the stabilization of co-active retinal ganglion cell synapses on the same post-synaptic membrane. In order to identify other molecular components of this activity-dependent mechanism we are using two-dimensional gel technology to analyze differences in the protein composition of normal and striped tecta, and molecular changes induced by chronic APV and NMDA treatment. Preliminary results indicate a number of changes in both cases, including an electrophoretic shift in an acidic protein of approximately 110 kD in APV versus NMDA treated animals and a decrease in an acidic protein of approximately 30 kD in the doubly versus singly innervated tecta. Our future investigations will utilize our ability to separate pre- and post-synaptic components by examining ^{35}S labelled tectal proteins subsequent to intraocular or intraventricular injection.

In addition, we have shown that antibodies to several cytoskeletal proteins specifically implicated in process stability (e.g. MAP-2, fodrin and tau), react specifically on western blots of larval tecta and we should be able to determine whether any of these molecular species are altered with double innervation or drug treatment of larval tecta.

Supported by grants NIH EY 06039 and NIMH MH 18397.

477.12

DISTRIBUTION OF ENKEPHALIN AND SEROTONIN-IMMUNOREACTIVITY IN THE OPTIC TECTUM OF THE COHO SALMON *ONCORHYNCHUS KISUTCH* CHANGES WITH AGE. E. Vecino*, P. Ekström* and S.O.E. Ebbesson (SPON: David Williams). Dept. of Zoology, University of Lund, Sweden, and Institute of Marine Science, University of Alaska Fairbanks, Alaska, USA.

In teleost fishes, the highly differentiated optic tectum is a center for the integration of sensory information. It has a concentric laminar organization with six strata: stratum marginale (SM), stratum opticum (SO), stratum fibrosum et griseum superficiale (SFGS), stratum griseum centrale (SGC), stratum album centrale (SAC) and stratum periventriculare (SPV) (Vanegas *et al., J. Comp. Neurol.* 154:43pp, 1974). Previous studies have indicated that the retinofugal projections undergo a reorganization during the time of smolt transformation, and that changes in the central serotonergic system occur during aging in the coho salmon. We have investigated the distribution of serotonin-immunoreactive (5HTir) elements in the optic tectum in pre-smolt, post-smolt and spawning coho salmon (*Oncorhynchus kisutch*), and compared it with the distribution of enkephalin-immunoreactive (ENKir) elements. ENKir neurons occur in the 5HTir nuclei of the upper brainstem (unpublished observations) that are the most probable sources of the 5HTir axons innervating the optic tectum. Antibodies against serotonin, Leu-enkephalin and Met-enkephalin were applied to cryostat sections of brains, fixed by perfusion with 4% paraformaldehyde and 0.25% picric acid in 0.1 M phosphate buffer (pH 7.4). Immunoreactivity was detected by the PAP method, and visualized with diaminobenzidine and hydrogen peroxide. A detailed study of distribution of immunoreactive elements was made in the median, dorsolateral and ventrolateral areas of the optic tectum at rostral, intermediate and caudal levels. The changes can be summarized (- no immunoreaction; +, ++, +++ immunoreactive axons, increasing densities):

	ENKir			5HTir		
	Pre	Post	Spawner	Pre	Post	Spawner
SM	-	-	-	-	-	-
SO	++	++	+++	-	-	-
SFGS/SGC	++	+++	+	+	+	+
SAC	++	++	++	+++	++	++
SPV	+	++	+	-	-	-

[Supported by N.I.H., Sea Grant (S.O.E.E.) and the N.P.R., Sweden (P.E.)]

477.13

GROWTH AND ARBORIZATION OF CHICK RETINOTECTAL AXONS. Kohji A. Matsui and Dennis D.M. O'Leary (SPON: S. Goldring), Dept of Neurosurgery, Washington Univ Sch of Med, St. Louis, MO 63110

Peripheral temporal retinal axons in chicks initially mistarget along the two main axes, medial-lateral (M-L) and rostral-caudal (R-C), of their major target, the contralateral optic tectum. We report here that axons from other parts of chick retina behave similarly. Small areas of retina (≤ 0.1%) were labeled with DiI in embryos aged E10-E19 when fixed, and whole mounts of retina and tectum made. In the mature projection, labeled peripheral nasal axons project to a terminal zone (TZ) at the caudal tectal border, but are distributed across most of the M-L tectal axis more rostrally, indicating that they made major chnages in position as they grew across the tectum. Developing nasal axons occasionally form transient branches and arbors in rostral tectum. After maturation, axons labeled from peripheral dorsal retina at its nasal-temporal midline project to a TZ in far lateral tectum, near its R-C midline. Earlier injections label axons that enter tectum at its lateral edge, but spread uniformly over the lateral third of tectum. Some axons enter tectum far medially; a few are found in between. As the axons grow caudally, most grow past their TZ, some continuing to the caudal border. Branches and arbors form rostral and caudal to the TZ. After maturation, axon trajectories indicate that many make course corrections to enter the TZ, but most surviving axons are found within 500 um of the TZ. Those seen earlier medially are not present. Injections in peripheral ventral retina label a mirror-image pattern to that seen for dorsal injections. After maturation, injections in central retina label a TZ in central tectum, but again many axons make trajectory changes along the M-L and R-C axes to achieve their TZ. At earlier stages, many central axons also overshoot their TZ, but do not grow distantly into caudal tectum; typically branches and arbors form along their length. Thus, central retinal axons which reach the tectum early, as well as late arriving axons from peripheral retina, initially mistarget along the M-L and R-C tectal axes, but often correct these errors, attain their appropriate TZ, and survive.

477.14

REBOUND ACTIVITY IN GOLDFISH OPTIC NERVE IS ASSOCIATED WITH THE POLYSYNAPTIC FIELD POTENTIAL IN OPTIC TECTUM. W. M. King and J. T. Schmidt. Dept. Biol. Sci., SUNY-Albany, NY 12222.

In an in vitro preparation of goldfish optic tectum and full length optic nerve, shocking the nerve leads to an orthodromic compound action potential (CAP) in the nerve and a monosynaptic field potential (FP) in tectum, followed by a polysynaptic FP 12-16msec later. Associated with the polysynaptic FP is a rebound CAP recorded from the end of the nerve at a latency of 20-25msec. That this second CAP was of tectal origin was indicated by its long latency and its disappearance after crushing the optic tract central to the stimulating electrodes. In addition, manipulations that block the long latency polysynaptic FP in tectum also block the retinopetal CAP in the optic nerve. When the long latency polysynaptic FP was blocked by 1-5 μM carbachol, alcuronium, or curare, by high [Mg] or by increasing stimulus frequency, the retinopetal CAP in the optic nerve was also eliminated. The rebound CAP could either be due to the activation of tectal efferent fibers to the retina or to the 'backfiring' of many optic fibers, i.e., antidromic activity originating at retinal terminals. The amplitude, often 50% of the initial orthodromic CAP, suggests backfiring since efferents to the retina are very few in number. In addition, double stimulus CAP collision experiments also suggest "backfiring". The retinal terminals are known to have nicotinic acetylcholine receptors (Henley et al., Science 1986), and thus could be effectively depolarized by acetylcholine release onto them from cholinergic terminals of tectal neurons. This hypothesis is consistent with the fact that cholinergic agonists and antagonists have been found to modulate retinotectal synaptic transmission, and suggest that this effect on the terminals can be quite dramatic. Supported by NIH grant EY-03736.

477.15

ACTIVITY SHARPENS THE REGENERATING RETINOTECTAL PROJECTION IN GOLDFISH: EFFECTS OF STROBE AND TTX ON OPTIC ARBOR MORPHOLOGY. John T. Schmidt, Dept Biol Sci, SUNY-Albany, NY 12222.

Activity was either blocked with tetrodotoxin (TTX) or synchronized with a strobe light (1Hz) in regenerating projections from 2 to 8 weeks postcrush. HRP stained arbors were drawn by camera lucida from tectal whole mounts (62, 52 and 33 arbors regenerated under TTX, strobe and control conditions) and compared to 118 normals. Control arbors at 8 weeks postcrush were roughly normal in appearance: they arose from the same three calibers of axons (fine, medium and coarse), were associated with the same three sizes of arbors (small- 128μm, medium- 212μm and large- 275μm avg extent), had about 16 vs the normal 21 branches and terminated at the same depths. However, they were on average 16% larger in hz extent. TTX and strobe regenerated arbors at 8 weeks were 71% and 119% larger on average than the control regenerates, but had approximately the same number of branch endings. In fact, all three classes were larger and abnormal in appearance. Thus, the one significant effect of manipulating activity was to enlarge the spatial extent of the arbors, as expected since these treatments prevent the sharpening of the retinotopic map as assessed electrophysiologically. Nonregenerating projections undergoing similar TTX and strobe treatments were essentially normal but showed slight enlargement (23 and 38% each). Since Schmidt et al. (1988 J.Comp.Neurol 269:565-591) previously showed that regenerating axons initially make widespread branches and later retract many of those branches, the present findings support the idea that blocking activity or synchronizing activity interferes with the elimination of some of the errant branches and with the focussing of branches into a single cluster. (Supported by NIH grant EY-03736).

477.16

FETAL EYE AND SCIATIC NERVE BRIDGES BETWEEN EYE AND TECTUM. H.F.Lowe, B.H.Hallas, G.Jacobsen, M.A.LaCorte, S.P.Lee, S.D.Loughlin and M.F.Zanakis*. (SPON: M.Wells). New York College of Osteopathic Medicine, Old Westbury, N.Y. 11568 and *American Bio-Interface Corporation, New York, N.Y. 10276.

Eighteen day embryonic rat eyes (E18) or postnatal day 1 eyes (PN-1) were surgically dissected and placed in lacated Ringers solution. Simultaneously, a 3 cm segment of adult rat sciatic was removed and either an E18 or PN-1 eye was sutured to the distal end with 10-0 suture. The fetal eye/sciatic nerve bridge complex was inserted into an adult host eye from which the lens was removed. In addition, the host's optic nerve was completely transected 2 mm distal to the orbit. The proximal end of the sciatic nerve bridge was inserted through a burr hole in the cranium into the contralateral superior colliculus. Thirty to one hundred and eighty days post implantation of the fetal eye/sciatic nerve bridge, a 20% solution HRP was either injected intraocularly or into the contralateral superior colliculus. Forty-eight to ninety-six hours after injection animals were sacrificed. In both age groups, (E18 or PN-1) anterograde and retrograde HRP label demonstrated axonal connections between implanted eye and superior colliculus and both age eyes developed a laminar arrangement reminiscent of the normal adult rat eye. Research supported in part by the Riland Neuromuscular Institute.

477.17

AFFERENT /TARGET MISMATCHES IN THE MAMMALIAN RETINOTECTAL SYSTEM ARE BALANCED BY CHANGES IN RETINAL AXON ARBORIZATION AND PROJECTION PATTERNS. B.L. Finlay and S.L. Pallas. Dept. of Psychol., Cornell University, Ithaca, NY 14853 and Dept. of Brain & Cog. Sci., M.I.T., Cambridge, MA 02139.

The receptive field size of individual hamster optic tectum cells is unaffected by increases in afferent/target ratio (Pallas & Finlay, Vis. Neurosci. 2:121, '89), suggesting a lack of change in convergence between retinal cells and single tectal cells. The present study was designed to determine whether this is accomplished by afferent arbor reduction, projection to alternate targets, or both.

Afferent/target size mismatches were made in hamsters by unilateral partial deletion of the optic tectum on the day of birth. Equivalent HRP injections were made into lesioned and unlesioned tecta in adults. We then determined the total number, density, and extent of backfilled retinal somata.

We found that for small lesions (20-50%), the total area of retina labelled and the number of backfilled retinal ganglion cells was larger than normal, but cell density was unchanged. For larger lesions, the maximum density and total number of backfilled cells per injection declined markedly, but total area labelled was larger. These results suggest that for small reductions in target size, ganglion cells from a larger area of retina project to each tectal volume, thereby reducing their arbor to maintain receptive field size. For larger target deletions, projection to alternate targets also occurs. For extreme lesions, only a partial topographic map is formed (Finlay et al., Nature 280:153, '79; Pallas & Finlay, Vis. Neurosci. 2:121, '89).

These changes in arborization pattern preserve receptive field properties over at least a twofold increase in convergence ratio. The same mapping constraints could provide a buffer for normal variations in afferent populations, and could function in aligning topographic maps with differing numbers of afferents.

477.18

NMDA RESTORES PLASTICITY OF IPSILATERAL TECTAL MAPS IN POST-CRITICAL PERIOD XENOPUS. W.J. Scherer* & S.B. Udin. Dept. of Physiol., SUNY, Buffalo, NY 14214.

The tectum of Xenopus frogs receives input from both eyes. The contralateral eye's projection reaches the tectum directly, via the optic nerve, and the ipsilateral eye's projection reaches the tectum indirectly, via the nucleus isthmi. The ipsilateral map shows great plasticity during development and it will reorganize in response to rotation of either eye, but this plasticity normally ends by about 3 months after metamorphosis. The process by which the ipsilateral map comes into register with the contralateral map involves matching of visually-elicited activity, and we have shown that normal function of the NMDA-type glutamate receptor is essential to this matching process.

We have tested whether chronic application of N-methyl-D-aspartate (NMDA) can restore the ability of isthmotectal axons to shift their connections in 8-month post-metamorphic frogs. The left eye was rotated 90° clockwise. Slabs of slow-release elvax polymers impregnated with 0.1 mM NMDA were inserted under the pia of the right tectal lobe in half of the frogs; the other eye-rotated frogs served as controls. After a survival period of 3 months, the contralateral and ipsilateral maps to the right tectum were recorded. In control frogs, the two maps were misaligned by 90° with respect to each other, and the ipsilateral fields often were weak. In contrast, in the NMDA-treated frogs, most ipsilateral receptive fields were in register with the contralateral receptive fields recorded at the same sites.

We thus conclude that plasticity in this system can be restored by chronic application of NMDA. This result implies that the duration of ipsilateral plasticity during development is normally limited by some change in the NMDA receptor or by processes triggered by activation of the receptor.

Supported by USPHS Grant EY-03470 to S.B.U.

477.19

TEMPORAL PROFILE OF THE "CRITICAL PERIOD" FOR INTERTECTAL PLASTICITY IN XENOPUS LAEVIS: RELATION TO NORMAL DEVELOPMENTAL DEMAND AND EXTENSION BY DARK-REARING. S.Grant* & M.J.Keating* (SPON: Brain Research Association) National Institute for Medical Research London NW7 1AA UK

A commissural system of intertectal connections mediates binocular visual integration in Xenopus. During development, this system displays plastic adjustments in response to marked changes in eye alignment which commence at metamorphosis and continue at a reducing rate for some months thereafter. This normal plasticity utilizes visual experience. The plastic capacity of the system may be revealed by challenging it to adjust to surgical eye rotation. The system shows a high plastic capacity (ability to respond to large eye rotations) at metamorphosis and a progressive reduction in capacity over a 3-month period after it. The profile of this capacity mirrors temporal features of the normal changes in eye alignment. Visual experience is not only utilized to effect intertectal plasticity but also contributes to its age-related reduction. Animals deprived of vision during the normal critical period, and indeed for an extended time after it, show the high plastic capacity normally only seen in metamorphosing animals.

477.20

MODIFICATIONS IN THE DISTRIBUTION OF NEUROACTIVE SUBSTANCES IN DEVELOPING VISUAL STRUCTURES OF THE PIGEON BRAIN. P.Bagnoli, J.T.Erichsen, G.Fontanesi* and R.Alesci*. Dept. of Physiol. Biochem., Pisa Univ., I-56100 and Dept. of Neurobiol. and Behav., SUNY, Stony Brook, NY 11794.

We analyzed visual regions immunocytochemically 1 to 9 days posthatching to localize perikarya and neuropil containing choline acetyltransferase (ChAT), glutamic acid decarboxylase (GAD), GABA, substance P (SP), neuropeptide Y (NPY), serotonin (5HT) and tyrosine hydroxylase (TH). Most labeled neurons displayed complex modifications in their pattern of distribution between 3 and 6-9 days, when they were innervated by visual afferents and attained adultlike cytoarchitecture. Major changes can be summarized as follows: 1) some neuronal populations present at hatching disappeared towards adulthood (e.g. GABA and GAD in parvicellular n. isthmi, Ipc; SP in optic tectum, TeO); 2) changes in the density and distribution of neurons and processes (e.g. progressive laminar segregation within layers of TeO) and 3) changes in neuronal morphology (e.g. ChAT cells in Ipc and TeO; NPY cells in n. pretectalis). These findings indicate that neuronal populations in the visual regions examined undergo modifications of their morphology and transmitter/peptide phenotypes when innervated by their afferents. Grants: CNR 880205004 to PB and EY0458 to JTE.

EPILEPSY: EXCITATORY AMINO ACIDS

478.1

ROLE OF NMDA AND NON-NMDA RECEPTORS IN PICROTOXIN-INDUCED EPILEPTIFORM ACTIVITY IN RAT NEOCORTEX. W.L. Lee* and J.J. Hablitz (SPON: P. Kellaway). Neurobiology Research Center, Univ. of Alabama at Birmingham, Birmingham, AL 35294.

D-2-amino-5-phosphonovaleric acid (D-APV) and 6-cyano-2,3-dihydroxy-7-nitro-quinoxaline (CNQX) are antagonists of NMDA and non-NMDA excitatory amino acid receptors, respectively. The involvement of these two classes of receptors in generation of picrotoxin-induced interictal discharges in the adult rat neocortex was examined.

D-APV (20 μM) reduced the amplitude of the PDS by 15±3% (n=8). PDS duration (measured at 50% of peak amplitude) was reduced by 36±8% (n=7). Complete suppression of PDSs was never achieved with D-APV. CNQX (5 μM) had no effect on the peak amplitude of the PDS in 83% (n=6) of the cells tested but delayed the onset of the PDS (n=8) and reduced its duration by 56±10% (n=6). In four cells, CNQX was able to abolish evoked epileptiform activity. Control PDSs were shorter in these neurons than in cells where PDSs were not blocked by CNQX (42±9 vs 190±20 ms). PDSs were always abolished when D-APV and CNQX were applied together, suggesting that an NMDA-mediated component is unmasked in the presence of CNQX. These results indicate that both NMDA and non-NMDA receptors are activated during PDSs in the rat neocortex. NMDA receptors contribute to the PDS but are not necessary for its generation. Non-NMDA receptor antagonists are capable of completely blocking epileptiform activity. Supported by NS18145 and NS22373.

478.2

KINDLING LIKE INDUCTION OF ELECTROGRAPHIC SEIZURES IN VITRO INCREASES ECTOPIC ACTION POTENTIAL GENERATION THROUGH NMDA RECEPTOR-DEPENDENT MECHANISMS. W. A. Wilson and S. F. Stasheff. Depts. of Pharmacology and Medicine, Duke Univ. and V.A. Medical Centers, Durham, N.C.

We have previously described an in vitro model of kindling-like epileptogenesis in which electrographic seizures (EGSs) are induced in rat hippocampal slices through N-methyl-D-aspartate (NMDA) receptor-dependent mechanisms. EGS induction is accompanied by a marked increase in the occurrence of "baseline spikes," action potentials which arise sharply from the baseline membrane potential, and appear to be generated from an "ectopic" site distant from the soma. Here we report that this increase is also NMDA receptor-dependent.

EGSs were induced in rat hippocampal slices with repeated stimulus trains. In parallel, CA3 pyramidal cells displayed a marked increase in the frequency of baseline spike firing (Neurosci. Abstr. 1989). In an additional 12 experiments, cells were continuously recorded in the presence of the NMDA receptor antagonist D-APV (100 uM). APV prevented EGS induction in all cases, and the increase in baseline spiking in 9. In one case, APV allowed a mild enhancement of afterdischarges; in parallel, baseline spikes began to fire at a low frequency (0.4/min.). Upon washout of APV, afterdischarges developed into full EGSs, and the frequency of baseline spikes increased markedly (>20/min.). Thus, EGS induction and increased ectopic action potential generation were both dependent on NMDA receptor activation.

478.3

Epileptiform bursts induced by 4-aminopyridine (4AP) in the rat hippocampus: possible mechanisms. P. Perreault and M. Avoli MNI, McGill University, Quebec, Canada, H3A-2B4.

Disinhibition and NMDA receptor activation have been considered as important factors in epileptogenesis. Here we have used conventional intra- and extracellular recordings to study in the CA3 region of rat hippocampal slices the physiological bases of 4AP-induced activity. Intracellular recordings with QX-314-filled microelectrodes showed that the spontaneous field bursts (SFBs) were associated with 22-38 mV giant EPSPs. Both SFBs and giant EPSPs were insensitive to NMDA antagonists (CPP and APV), but were blocked in a dose related way by CNQX and DNQX (3-10μM). Under these conditions, BMI sensitive, spontaneous and evoked IPSPs were seen. There was a strong correlation between non-synchronous EPSPs and SFBs: (1) during the interburst interval they appeared 3-5 times more numerous in the 50ms preceding the burst onset; (2) there was a linear correlation between EPSPs and SFBs frequency (n=10 cells); (3) The sensitivity of EPSPs and SFBs to CNQX and DNQX was similar (IC50 1.5μM). We conclude that SFBs induced by 4AP do not require disinhibition or NMDA receptor involvement.

478.4

SUSTAINED ANTICONVULSANT ACTION OF 3-(2-CARBOXY-PIPERAZIN-4-YL)-1-PHOSPHONATE (CPP-ENE) FOLLOWING I.V. OR ORAL ADMINISTRATION IN BABOONS. S. Patel* and B.S. Meldrum. (SPON: Brain Research Association). Dept.of Neurology, Inst. of Psychiatry, London, SE5 8AF, U.K.

CPP-ene is an unsaturated analogue of 3-(2-carboxypiperazin-4-yl)-propyl-1-phosphonate (CPP) that has been shown to be more potent than CPP as an NMDA antagonist in vitro and to be orally active in the rat electroshock test (Herrling et al., this meeting). CPP-ene was administered either intravenously (0.5-16 mg/kg) or orally (8-64 mg/kg) to baboons, Papio papio, with photosensitive epilepsy. CPP-ene, 8 mg/kg, i.v. produces within 15 min a complete suppression of photically-induced myoclonic responses that lasts 24-48h. Oral administration of CPP-ene, 32-64 mg/kg, leads to suppression of responses after 24h, with recovery after 72-96h. Analysis of plasma samples indicates a rapid clearance of CPP-ene after i.v. or oral administration. Neurological side effects were seen after 16 mg/kg i.v. but not after 64 mg/kg orally. These properties indicate a potential for clinical use in epilepsy.

478.5

BACLOFEN-INDUCED EPILEPTIFORM ACTIVITY IS REDUCED BY THE NMDA RECEPTOR ANTAGONIST D-2-AMINO-5-PHOSPHONOVALERIC ACID (APV) IN RAT DENTATE GYRUS. E. C. Burgard and J. M. Sarvey (SPON: G. Decker). Dept. of Pharmacology, Uniformed Services University of the Health Sciences, Bethesda, MD 20814.

The actions of baclofen in the central nervous system are mediated through $GABA_B$ receptors. Activation of presynaptic $GABA_B$ autoreceptors located on GABAergic nerve terminals can result in an inhibition of GABA release, leading to a net disinhibition of responses. We have examined the effects of D(-)APV on the disinhibition of evoked responses produced by (-) baclofen in the rat dentate gyrus.

Rat hippocampal slices were maintained in an interface recording chamber. Extracellular population spikes were recorded in the granule cell layer of the dentate gyrus in response to medial perforant path stimulation. A 15 min bath application of baclofen produced a dose-dependent increase in the amplitude of the initial evoked population spike (mean 202% of control at 5μM) that could be blocked by phaclofen (200μM, mean 107%). Effects of baclofen lasted through a 30 min wash period. At a concentration of 5μM, baclofen also induced epileptiform activity as indicated by the occurrence of multiple (2-4) population spikes, and a loss of paired pulse inhibition at interpulse intervals of 20-30 msec. This activity was also specific to the dentate gyrus, as population spike amplitudes recorded in field CA3 were reversibly depressed by baclofen (mean 50% of control). A 15 min bath application of APV (10μM) produced a depression of the evoked response (mean 71%). When APV was applied for 15 min before, during, and after baclofen (5μM), the occurrence of multiple population spikes was reduced (1-2). In addition, APV could reverse baclofen-induced epileptiform activity. We hypothesize that baclofen acts primarily presynaptically in the dentate gyrus to inhibit GABA release, and the resulting epileptiform activity is APV sensitive. These findings further support the potential usefulness of NMDA receptor antagonists as antiepileptic agents. (Supp. by NIH grant NS23865)

478.6

THE EFFECTS OF 7-CHLOROKYNURENIC ACID, A STRYCHNINE INSENSITIVE GLYCINE RECEPTOR ANTAGONIST, ON THE DEVELOPMENT OF AMYGDALAR KINDLING IN RATS. D.L. Yourick*, M.A. Bixler and J.L. Meyerhoff (SPON: S.P. Sparenborg). Dept Med Neurosci, Walter Reed Army Inst. Res., Washington, DC 20307-5100.

Glycine (Gly) has been shown to be an absolute requirement for NMDA receptor activation (Kleckner, NW and Dingledine, R, Science 241:835-837, 1988). 7-chlorokynurenic acid (7CKYA) has been shown to be the most potent and selective antagonist of the Gly-modulatory receptor of the NMDA receptor complex (Kemp, JA et al., PNAS 85:6547-6550, 1988). Kynurenic acid (KYA) has some Gly receptor antagonist properties, is also a weak competitive excitatory amino acid receptor antagonist (Birch, PJ et al., Eur J Pharmacol 154:85-87, 1988) and antagonizes amygdalar kindling in rats (Dennison, Z and Cain, DP, Soc Neurosci Abstr 13:761, 1987; Thompson, JL et al., Epilepsy Res 2:302-308, 1988). We sought to evaluate the effect of 7CKYA on kindling development. 7CKYA was given icv (10, 18 and 32 ug in 3 uL) 1 hr before amygdalar kindling stimulation (200 uamp, biphasic, base to peak). Stimulations were continued until five Stage 5 convulsions were obtained. The number of stimulations to first Stage 3, first Stage 5 and last Stage 5 convulsion was not different in untreated, vehicle-treated and all drug-treated groups. AD duration was not significantly different in untreated, vehicle-treated and all 7CKYA-treated groups. 7CKYA had little or no effect on kindling development despite structural and functional similarities to KYA.

478.7

THE INFLUENCE OF 1-HYDROXY-3-AMINOPYRROLIDONE-2 (HA-966) ON KINDLED SEIZURES IN THE RAT. M.A. Bixler, J.L. Meyerhoff and D.L. Yourick. Dept. of Medical Neurosciences, Walter Reed Army Institute of Research, Washington, D.C. 20307-5100.

Glycine has been found to be a requirement for NMDA receptor activation (Kleckner, N.W. and Dingledine, R., Science 241:835-837, 1988). The NMDA receptor antagonist HA-966 appears to compete with glycine at the glycine modulatory site of the NMDA receptor complex (Lodge, D. In: Cavalheiro, E.A. et al. (eds.) Frontiers in Excitatory Amino Acid Research, in press). Another agent, kynurenate, which is also an excitatory amino acid receptor and strychnine-insensitive glycine receptor antagonist, has been shown to antagonize amygdalar kindling in rats (Dennison, Z. and Cain, D.P., Soc. Neurosci. Abstr. 13:761, 1987; Thompson, J.L. et al., Epilepsy Res.. 2:302-308, 1988). To evaluate the effect of HA-966 on kindled seizures, male Sprague-Dawley rats were implanted with bipolar electrodes in the basolateral amygdala (AP -2.2 mm, Lat +4.7 mm, Vent +8.5 mm), and a cannula (AP -1.0 mm, Lat -1.6 mm, V -3.5 mm) in the lateral ventricle. Beginning 7 days post-surgery, subjects received daily stimulation (1.0 sec train, 1 msec biphasic pulses, 60 Hz, 200 microamps base-to-peak) until a criterion of five cumulative stage 5 seizures was obtained. A 30 minute pretreatment with HA-966 (100 or 200 microgm in saline given to fully-kindled rats, i.c.v.) failed to significantly alter seizure score or afterdischarge duration relative to vehicle-injected controls. Despite a similar pharmacological profile to kynurenate, HA-966, at a dose which induced occasional sedation and catalepsy prior to amygdalar stimulation, did not effect kindled seizures in the rat.

478.8

DEXTRORPHAN INHIBITION OF PENICILLIN-INDUCED EPILEPTIFORM DISCHARGES IN RAT HIPPOCAMPAL SLICE, J. Aryanpur*, A.E. Cole, C.U. Eccles, R.S. Fisher, Departments of Neurology and Neurosurgery, The Johns Hopkins University School of Medicine, and Department of Pharmacology, University of Maryland School of Pharmacy, Baltimore, MD 21205

We studied the anticonvulsant effect of dextrorphan (DX), a metabolite of dextromethorphan with NMDA antagonist properties, against penicillin-induced epileptiform bursting. Extracellular and intracellular recordings were performed in pyramidal neurons from region CA1 of the rat hippocampal slice. In control perfusate a single field population spike was evoked by afferent electrical stimulation. Stimulation after addition of 3.4 mM penicillin (PCN) to the slice perfusate produced 5 or 6 population spikes, reflecting repetitive synchronous firing of pyramidal cells. DX doses of 1-100 μM were applied to 47 slices. Concentrations of 100 μM DX (n=10) decreased the mean number of population spikes to 61.5 ± 2.0% of control, decreased the mean summed amplitude of the 2nd-6th spikes to 67.4 ± 4.2% of control, and usually abolished the last three population spikes ($p<0.05$). Depression of the evoked field was maximal at 40 minutes after start of DX perfusion, and rarely reversed with 90 minute wash.

The dose-response curve of DX was biphasic. Perfusion with 10 μM DX increased the mean summed amplitude of all population spikes to 128.7 ± 17.6% of control (n=10, $p<0.05$). This enhancement was more pronounced with the 2nd-6th population spikes.

Intracellular recordings (n=6) showed that DX 100 μM did not affect mean cell RMP, input resistance, or response to direct depolarizing current. In contrast, 100 μM DX decreased the amplitude of the electrically-evoked, penicillin-induced paroxsysmal depolarization shift (PDS) to 60 ± 8% of control ($p<0.05$).

PCN induces slice epileptiform activity by suppressing GABAergic inhibitory mechanisms. The ability of DX to suppress this non-NMDA mediated epileptiform activity confirms its potential usefulness in the treatment of seizures. Caution is warranted, however, in view of a possible enhancement of epileptiform activity at low doses.

478.9

DEXTROMETHORPHAN (DM) EVOKES EPILEPTIFORM EEG ACTIVITY AND BEHAVIORAL CONVULSIONS IN RAT. D.F. Heard*, V.S. Westerberg* and D.M. Feeney (SPON: G.Hodge). Depts. of Psychol. and Physiol., U. New Mexico, Albq., NM 87131.

DM attenuates some manifestations of evoked seizures in rats and has been suggested for study of its anticonvulsive potential in epileptic patients (Neurosci. Lett. 86:340,1988). However, we observed that DM evokes convulsions accompanied by epileptiform EEG in normal adult male rats. DM (35, 50 or 75mg/kg;i.p.) or saline was given every 48 hr for 10 days to 19 rats, 8 with implanted electrodes. By 1 hr after the second administration of 75mg/kg, 3-4 convulsions occured in all rats, some dying after a convulsion. At 50mg/kg most rats did not convulse until the third administration. These convulsions consisted of head nodding, body rigidity often with tail extension, without loss of balance and occasionally slight rearing accompanied by alternating forelimb movements. Convulsions were accompanied by high voltage synchronous EEG and post-ictal depression. Stereotypic behaviors characterized by alternating forelimb movements, often with lateral head movements but without EEG abnormalities were also observed. After each 35mg/kg dose, stereotypic behaviors, but not convulsions were seen. These data indicate that the proposed NMDA antagonist DM has convulsant actions depending on dose and perhaps species. Thus, caution is warranted prior to clinical anticonvulsant trials. Supported by DHHS R01 NS20220-03.

478.10

ANTICONVULSANT ACTIVITIES OF 1-PHENYLCYCLOHEXYLAMINE ANALOGS. S. Yamaguchi, A. Thurkauf*, B.R. de Costa*, K.C. Rice and M.A. Rogawski. Medical Neurology Branch, NINDS and Section on Drug Design and Synthesis, NIDDK, NIH, Bethesda, MD 20892.

We previously reported that 1-phenylcyclohexylamine (PCA), like its analog the dissociative anesthetic phencyclidine (PCP), is a potent anticonvulsant in the maximal electroshock (MES) test. However, in contrast to PCP, PCA fails to cause motor impairment at anticonvulsant doses (M.A. Rogawski *et al., J. Pharm. Exp. Ther.* **249**, in press). In the present study, we determined the activities of 38 PCA analogs in the mouse MES seizure and horizontal screen motor toxicity tests. Our analogs had the PCA nucleus modified in the following ways: (i) methyl, methoxy, fluro, trifluromethyl, thiomethyl, phenyl, benzyloxy or 2-napthyl substitutions on the phenyl ring, (ii) methylations of the amino nitrogen, (iii) modifications in the alkyl ring size. In addition, we examined all stereochemically possible cyclohexane ring methylated PCA derivatives. The ED_{50} values for protection against MES seizures of the compounds ranged from 4.6 to 40 mg/kg whereas the TD_{50} values in the toxicity test ranged from 1.6 to 78 mg/kg. There was a wide variation in the therapeutic indexes ($TI=TD_{50}/ED_{50}$) of the compounds (0.34-3.5; PCA, 2.3). In fact, even those compounds with similar ED_{50} values often had widely divergent TI values. The compounds with the highest TI values were *cis*-4-methyl-PCA and phenylcyclopentylamine. We conclude that certain analogs of PCA have a substantially enhanced therapeutic ratio compared with PCA. These analogs may provide a basis for the development of PCP-related anticonvulsants that share PCP's potent antiseizure activity but have less toxicity.

478.11

KETAMINE PROTECTS AGAINST BRAIN DAMAGE FROM PILOCARPINE SEIZURES IN ENTORHINAL-KINDLED RATS. D.G. Fujikawa, C.G. Wasterlain, C. Yang*, and K. Thompson*. V.A. Med. Ctr., Sepulveda, CA 91343 and Dept. of Neurology, UCLA Sch. of Med., Los Angeles, CA 90024.

The noncompetitive N-methyl-D-aspartate (NMDA) antagonist ketamine may be neuroprotective in global ischemia. We studied its effect on the brain damage produced by pilocarpine (PC) seizures in kindled SD rats. After entorhinal (ER) kindling and a rest period of at least 2 wks, half the rats received ketamine, 100 mg/kg i.p., 15 min prior to PC; all were given PC, 200 mg/kg i.p., and had 3 h seizures which were stopped with atropine, diazepam and phenytoin. Brain perfusion-fixation was performed 3 d later. The degree of neuronal necrosis was evaluated on a 0 to 3+ scale.

Rats without ketamine showed neuronal damage of 2-3+ in piriform and ER cortex, 1-3+ in amygdala and CA1-4 of hippocampus, 1-2+ in dentate gyrus and thalamus, and 1+ in cerebral cortex and septal nuclei (n=3). Despite having continuous EEG spike-wave discharges for 3 h, rats with ketamine (n=3) had no neuronal necrosis except for 1 rat which showed unilateral 2+ damage to dorsal CA3-4 neurons in 7/13 sections (6/13 were normal). Besides showing that ketamine reduces seizure-induced brain damage, these results point to a dissociation between its neuroprotective and anticonvulsant effects and suggest that the damage may be NMDA-receptor-mediated.

478.12

EFFECT OF Ca^{2+}-CHANNEL BLOCKERS ON N-METHYL-D-ASPARTATE (NMDA)- AND QUISQUALIC ACID (QA)-INDUCED SEIZURES. H.S. White*, N.A. Singh*, E.A. Swinyard*, and R. Zobrist[1]. Dept. of Pharmacol. & Toxicol., Univ. of Utah, Salt Lake City, UT 84112 and [1]Marion Labs, Kansas City, MO 64137.

Intracerebroventricular (icv) administration of the glutamate agonists NMDA and QA to mice induces intense seizure activity culminating with forelimb tonic extension (FTE). The Ca^{2+}-channel antagonists flunarizine, diltiazem and its analogue TA3090 were examined for their ability to block FTE induced by icvNMDA and icvQA. Groups of 5 to 11 CF#1 mice were pretreated (i.p.) for 30 min with increasing doses of each agent and then challenged with the convulsive dose 97 of NMDA (3.0 µg/5 µl, icv) or QA (42 µg/5 µl, icv). Animals not displaying FTE within 30 min were considered protected. Flunarizine was the most potent against NMDA-induced FTE followed by diltiazem and TA3090 (ED50's: 3.52, 16.8 and 37.2 µmoles/kg, respectively). All three agents were effective against QA-induced FTE (ED50's: 9.6, 67.4, and 122 µmoles/kg for flunarizine, TA3090 and diltiazem); however, at doses of diltiazem producing complete protection, deficit by the rotorod test was observed. These results suggest that agents which block Ca^{2+} influx also block the excitatory effects of two glutamate agonists and support the further development of Ca^{2+} antagonists for the treatment of seizure disorders. (Supported by a grant from Marion Laboratories and NIH Contract NO1-NS-4-2361.)

478.13

INCREASE IN EXCITATORY AMINO ACID RECEPTOR-MEDIATED PHOSPHOINOSITIDE HYDROLYSIS IN THE AMYGDALA/PYRIFORM CORTEX OF DEEP PREPIRIFORM CORTICAL KINDLED RATS. K.Akiyama, N.Yamada*, A.Daigen*, H.Ujike* and S.Otsuki*. Dept. of Neuropsychiatry, Okayama University Medical School, Okayama 700, JAPAN.

We previously demonstrated that ibotenate (IBO)-stimulated phosphoinositide (PI) hydrolysis increased significantly in the right amygdala/pyriform cortex (AM/PC), which is contralateral to the stimulated site (left), four weeks after the last seizure in the AM kindled rats (Akiyama et al., Brain Res. 485:95-101,1989). Furthermore, we found such a significant increase four weeks after the last seizure in the AM/PC of the hippocampal (HIPP)-kindled rats (Yamada et al., Brain Res. in press, 1989). These findings strongly suggest that excitatory amino acid (EAA) receptor-mediated PI hydrolysis increases long-lastingly in the AM/PC independently of a primary stimulation site. The present study examined whether deep prepiriform cortical (DPC) kindling elicits similar changes. Electrodes were implanted into the left DPC (coordinate, AP:4.0, L:3.2, D:6.8) to prepare kindled rats. One week after the last seizure, IBO-stimulated accumulation of [^{3}H]inositol 1-phosphate significantly increased in the AM/PC but not HIPP or limbic forebrain including DPC. This result confirms our previous view that PI hydrolysis coupled to EAA receptors in the AM/PC may be associated with epileptogenesis which is transsynaptically induced in the secondary brain site in kindling.

478.14

ELEVATED NMDA RECEPTOR AND REDUCED GABA RECEPTOR BINDING IN HIPPOCAMPUS (HIP) FROM PATIENTS WITH TEMPORAL LOBE EPILEPSY (TLE). M.V. Johnston, J.W. McDonald, T. Hood*, C. Sackellares*, E. Garofalo*, P.E. McKeever, J. Troncoso & S. Gilman. Depts. of Neurology, Neurosurgery, & Pathology, University of Michigan, Ann Arbor, MI 48104 & Johns Hopkins University, Baltimore, MD 21205.

If excitatory (EAA) & inhibitory amino acid receptors contribute to the pathogenesis of TLE, then alterations in neurotransmitter receptor binding may be present in HIP resected from patients with TLE. We examined this hypothesis by measuring quisqualate & NMDA type EAA receptors, glycine & PCP receptors associated with the NMDA receptor complex, & GABA type A/benzodiazepine receptors in HIP resected from 8 patients with medically refractory TLE & from 8 age-matched post-mortem controls. Mean ages(yr±SEM) of TLE & control subjects were 32±5 & 46±5, respectively. Binding densities were measured in stratum moleculare(SMCA1) & stratum radiatum of CA1 (SRCA1), CA4, & stratum moleculare of dentate gyrus (SMDG). Statistical comparisons used ANOVA. Binding to the NMDA & the associated glycine sites were elevated by 20-100% in CA1 & SMDG of TLE HIP ($p < 0.01$), but unaffected in CA4. In contrast, binding to PCP receptors was reduced by 35-70% in all regions($p<0.001$). Binding to quisqualate receptors was unchanged in all regions except SMCA1 (63% increase, $p<0.01$). GABA type A & benzodiazepine receptor binding were reduced by 20-60% in CA1 & CA4($p<0.01$), but unchanged in SMDG. Alterations in EAA as well as inhibitory amino acid receptors may be involved in the pathogenesis of TLE.
Supported by J.H. Jackson EFA grant and NIH grant 15655.

478.15

REGIONAL BRAIN CONTENT OF AMINO ACID TRANSMITTERS IN GENETICALLY EPILEPSY-PRONE RATS (GEPR). S.M. Lasley, R. Burger*, J.W. Dailey and P.C. Jobe. Dept. of Basic Sci., U. of Ill. Coll. of Med., Peoria, IL 61656.

Regional brain amino acid levels were determined in moderate (G-3) and severe (G-9) seizure GEPR as well as in non-epileptic control animals. GEPR were divided into seizure-naive (SN) and seizure-experienced (SE) groups based on whether a seizure-inducing acoustical stimulus had been presented. SE were screened for seizures at 3 intervals at 45-60 days of age; SN were classified on the basis of seizures in littermates. Non-epileptic controls were similarly divided according to the occurrence or absence of exposure to the acoustical stimulus. Animals were sacrificed at 76±3 days of age and hippocampus (Hc), inferior colliculus (IC), striatum, thalamus and frontal and parietal cortices analyzed via PITC-amino acid derivatives. In Hc, aspartic (Asp) and glutamic (Glu) acids were significantly increased in G-9 vs. controls by 10-15%, while Asp was significantly decreased 10% in G-3 vs. controls. In IC, Glu and Asp were significantly elevated in SE vs. SN G-9. Changes in glycine content mirrored those in Glu in both regions. Increases in taurine compared to controls were striking in G-3 in both areas (57% in IC, 24% in Hc). These findings indicate that Glu/Asp activity may play a role in distinguishing seizure behavior between G-3 and G-9 GEPR. The import of the elevations in taurine is unknown.

478.16

BASAL AND NEWLY SYNTHESIZED (NEW) AMINO ACIDS (AA) IN MOUSE HIPPOCAMPAL SLICES AND THE EFFECTS OF POTASSIUM (K^+). W.D. Yonekawa, I.M. Kapetanovic and H.J. Kupferberg*. Epilepsy Branch, NINDS, NIH, Bethesda, MD 20892.

The balance between the effects of inhibitory (GABA) and excitatory [glutamate (GLU) and aspartate (ASP)] AA may be important in the pathogenesis and potential treatment of epilepsy. NEW AA may be more related to functional neuronal activity than the total tissue AA concentrations. Hippocampal slices from CR:NGP(S) mice were incubated in normal artificial CSF (4.4 mM K^+) in the presence of stable labeled precursor; glucose, alanine, pyruvate or acetate. The isotopic enrichment was used to measure NEW AA. Basal and NEW AA [GLU, GABA, ASP, glutamine (GLN) and alanine (ALA)] were quantitated by gas chromatography-mass spectrometry of the dimethyl t-butyl silyl derivatives. The ratio of NEW GLU/GLN was about 22 when precursors of the large GLU (neuronal) compartment were used and about 2 with precursors of the small GLU compartment (glial). In general, NEW AA were more sensitive than basal AA to the effects of elevated K^+ (16.4, 31.4 or 51.4 mM) and in some cases divergent effects were observed on basal and NEW AA.

478.17

EFECTS OF NMDA ON BASAL AND NEWLY SYNTHESIZED (NEW) AMINO ACIDS (AA) IN MOUSE HIPPOCAMPAL SLICES. I.M. Kapetanovic, W.D. Yonekawa and H.J. Kupferberg*. Epilepsy Branch, NINDS, NIH, Bethesda, MD 20892.

NMDA is an excitoxic agonist of glutamate (GLU) receptors which may play an important role in learning and memory processes, neurodegenerative disorders, cerebral ischemia and epilepsy. This study examined the effects of NMDA (0-500 uM) on AA neurotransmitters: GABA, GLU, aspartate and related AA. Hippocampal slices from CR:NGP(S) mice were incubated in artificial CSF in the presence of stable labeled precursor, $^{13}C_2$-alanine. The isotopic enrichment was used to measure NEW AA. Basal and NEW AA were quantitated by gas chromatography-mass spectrometry of the dimethyl t-butyl silyl derivatives. NMDA decreased basal and NEW GLU and increased basal and NEW GLN. Qualitatively similar effects of NMDA were observed when different labeled precursors were used (glucose, pyruvate or acetate). NMDA was effective in the presence of fluoroacetate, a selective inhibitor of the glial tricarboxylic acid cycle. The competitive NMDA antagonist, 2-APV, blocked NMDA effects. The results suggest that these effects of NMDA are neuronal and receptor related.

478.18

AMINO ACIDS IN DIALYSIS SAMPLES OBTAINED FROM NORMAL AND KINDLED RATS. M.Pintor*, S. De Mesquita, J.N. Crawley and N.S.Nadi(SPON: R.J.Porter). NINDS and NIMH, Bethesda Md. 20892

The levels of glutamate (GLU), glutamine (GLN), aspartate(ASP) and GABA were measured in dialysates from the ventral hippocampus contralateral to the electrode implanted side in kindled(K) and normal(C) rats. The measurements were made before, during and after exposure to veratridine (V)(50uM). In the absence of V no GABA was detected in the dialysates. In the presence of V the levels of ASP, GLU were increased over baseline by 1400% and 475% respectively in C and by 500% and 220% respectively in K. GLN was decreased by 85% of baseline in both C and K. GABA was detectable in both K and C only with V. The V stimulated release of GABA was not significantly different in K vs C. The basal and V stimulated levels of GLN were significantly lower in the K vs C. In the case of ASP the V stimulated levels were significantly lower in K vs C. The altered release patterns were reversed by removing V. The alteration in the levels of GLN and ASP in the kindled brain is suggestive of changes in the metabolism of excitatory amino acids in the ventral hippocampus of the kindled rat.

RECEPTOR MODULATION: UP AND DOWN REGULATION II

479.1

EFFECTS OF *IN VIVO* STEROID HORMONE TREATMENT ON *SIGMA* RECEPTOR BINDING IN THE FEMALE RAT BRAIN. F.Y. Ford-Rice*, M.D. Majewska and E.D. London (SPON: S.R. Cohen), Addiction Res. Ctr., NIDA, Baltimore, MD 21224.

Certain steroid hormones interact *in vitro* with *sigma* (σ) receptors in membranes from the brain and spleen. Progesterone (P) is the most potent, and estradiol (E) is inactive (Su, T.-P., et al., Science 240:219, 1988). We examined effects of P and E on σ receptor binding after hormone treatment *in vivo*. Female Fischer-344 rats were adrenalectomized and ovariectomized. The control group (I) had no hormone treatment; group II received s.c. P; group III, s.c. E; and group IV, E + P s.c., at physiological doses. Ligand binding to σ receptors in membranes from the cerebellum was measured using [^{3}H]haloperidol in the presence of 50 nM spiperone (to block catecholamine receptor binding). In control rats, the parameters of σ binding were: Kd = 5.7 ± 1.3 nM, Bmax = 1488 ± 321 fmol/mg protein. P treatment (48 h) markedly reduced the affinity of σ binding (Kd = 11.6 ± 2.1 nM; $p < 0.01$) concomitant with a tendency to increase receptor density (Bmax = 2711 ± 552 fmol/mg protein). E was ineffective *per se*, but it reduced the P-induced changes in σ binding. Thus, to some extent, P interacts *in vivo* with the σ receptors in a manner similar to that seen *in vitro*. However, increased densities of σ binding sites suggest the occurrence of compensatory up-regulation of σ receptors after *in vivo* P treatment.

479.2

MODULATION OF SIGMA RECEPTORS BY CHRONIC TREATMENT WITH SIGMA LIGANDS. M. Bremer*, L. Christine *, T.S. Rao and P. C. Contreras. (SPON: J. Monahan). G.D. Searle & Co., St. Louis, MO 63198

Due to suggestions that neuroleptics may induce dystonia through sigma receptors, and the finding that chronic haloperidol (HAL) treatment up-regulates phencyclidine (PCP) receptors, experiments were done to determine whether sigma or PCP receptors can be altered by chronic treatment with HAL or BMY-14802. Scatchard analysis of rat whole brain homogenates showed that chronic HAL or BMY-14802 did not alter the K_d or B_{max} of [^{3}H]-TCP, nor the K_d of [^{3}H]-(+)3-PPP. However, the B_{max} of [^{3}H]-(+)3-PPP was decreased by HAL and increased by BMY-14802 treatment. The behavioral potency of PCP was not altered by chronic treatments, but there was decreased potency of (+)SKF 10,047 in HAL-rats. The potency of BMY-14802 antagonism of apomorphine-induced climbing in mice was not altered by BMY-14802 treatment, but the potency of haloperidol was decreased. The difference in effect of HAL vs. BMY-14802 may be due to a) HAL is a sigma agonist or b) chronic D2 antagonism. In conclusion, chronic treatment with putative sigma antagonists modulated sigma, but not PCP, receptors.

479.3

SIGMA RECEPTOR AGONISTS MODULATE THE HYPOTHALAMIC-PITUITARY-ADRENAL (HPA) AXIS CENTRALLY. S. Mick*, V. Dilworth*, J. Michel, J. Farah, T.S. Rao, P.L. Wood, S. Iyengar, (SPON: H. Kim). CNS Diseases Research, G.D. Searle, St. Louis, MO 63198.

The effect of sigma receptor agonists on the hypothalamic-pituitary-adrenal (HPA) axis was evaluated in the rat. Adult male rats were injected interperitonially and sacrificed after 30 min. Trunk blood was obtained, the plasma separated, and plasma ACTH levels determined using RIA techniques. (+)-Pentazocine, a sigma agonist, was found to potentiate ACTH levels. The response was dose dependent and not reversed by naloxone (6 min pretreatment). Thus this effect did not appear to be mediated via opioid receptors. Similar results were seen using (+)-SKF 10,047 (NAAM): circulating ACTH levels increased significantly in a dose dependent manner and were not reversed by naloxone. Moreover, the effect was stereospecific since (-)-SKF 10,047 did not elicit a response. (+)-Pentazocine and (+)-SKF 10,047 were also evaluated using primary cultures of anterior lobe rat pituitary. Utilizing 7 day old cultures, concentrations of (+)-pentazocine and (+)-SKF 10,047 were applied which approximated the ED50 and 10xED50 from our sigma radioreceptor assay. Neither compound caused an increase in ACTH levels while corticotropin releasing factor (CRF), a positive control, caused a 5 fold increase over control. This implies that the increase in circulating ACTH levels is mediated within the central nervous system. It thus appears that sigma receptors modulate the HPA axis through a central mechanism.

479.4

SIGMA RECEPTORS MODULATE BOTH A9 AND A10 DOPAMINERGIC NEURONS IN THE RAT BRAIN. V. Dilworth*, S. Mick*, T.S. Rao, P. Contreras, P.L. Wood and S. Iyengar. CNS Diseases Research, G.D. Searle & Co., St. Louis, MO 63198.

(+)Pentazocine and (+)SKF-10,047 (NAAM) bind potently to sigma receptor sites, with Ki = 17 nM and 130 nM respectively in the [^{3}H]+PPP binding assay. The effects of these compounds on mesolimbic and nigrostriatal dopamine neurons were evaluated in rats. Male Sprague-Dawley rats were injected intraperitoneally with various doses of (+)pentazocine and (+)SKF-10,047 and the dopamine metabolites dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA) were measured by GC/MS. These compounds significantly increased DOPAC and HVA levels in the nigrostriatal (A9) and mesolimbic (A10) dopamine projections as measured in the striatum and olfactory tubercles, without significantly changing steady-state dopamine levels. These effects were stereospecific since
(-)SKF-10,047 did not alter dopamine metabolism. Naloxone did not reverse the effect of either compound, indicating that these increases are not mediated via opioid receptors.

Both compounds had no activity at either D1 ([^{3}H]SCH-23390 binding) or D2 ([^{3}H]spiperone binding) dopamine receptors in the striatum. These data suggest modulation of dopaminergic pathways by sigma receptors independent of dopamine receptor activity.

479.5

ACTIVATION OF PROTEIN KINASE C INCREASES THE RATE AND MAGNITUDE OF δ-OPIOID RECEPTOR DOWNREGULATION IN NG108-15 CELLS. S. Gucker and J.M. Bidlack. Dept. of Pharmacology, University of Rochester, Rochester, NY, 14642.

This study was undertaken to determine if protein kinase C (PKC) activation by phorbol 12-myristate 13-acetate (PMA) would alter the rate or magnitude of δ-opioid receptor downregulation in NG108-15 (NG) cells. Incubating NG cells with 30 nM PMA for up to 48 hr failed to induce δ-receptor downregulation. However, NG cells cultured with 1 nM etorphine and 30 nM PMA displayed evidence of δ-receptor downregulation greater than that obtained with etorphine alone. Culturing NG cells with 1 nM etorphine alone produced no downregulation with ≤ 3 hr of incubation, as measured by [^{3}H]DADLE or [^{3}H]diprenorphine binding to cell membranes. In contrast, culturing cells with 1 nM etorphine and 30 nM PMA reduced binding to membranes from these cells by 20-30% after 1 hr. The maximum effect of 1 nM etorphine and 30 nM PMA was reached at 6 hr, with binding reductions of > 50%. After 24 or 48 hr of treatment, a time period sufficient for PMA to downregulate PKC, the reductions of binding by etorphine with or without PMA were the same, approximately 20-30%. Scatchard analysis revealed that the PMA-induced decrease in binding was due to a reduction in the B_{max} value and not a change in affinity. The PMA enhancement of downregulation was blocked by naloxone or by substituting an inactive analog, 4α-phorbol, for PMA. These results suggest that PMA activated PKC can enhance opioid agonist-induced downregulation before the enzyme itself is downregulated.

479.6

CHARACTERIZATION OF THE EFFECT OF STEROID HORMONES ON STRIATAL D-2 DOPAMINE RECEPTORS. D. Lévesque and T. Di Paolo. Dept. of Molecular Endocrinology, Laval University Medical Centre, CHUL, Québec G1V 4G2 and School of Pharmacy, Laval University, Québec G1K 7P4, Canada

We have shown that estradiol (E_2) and progesterone (P) at physiological doses, acutely increase dopamine (DA) and its metabolite levels in the rat striatum. A dose of E_2 (100 ng, s.c.) also induces a conversion of the high into low agonist affinity states of the D-2 DA receptors 30 min after the steroid injection. This report investigates the *in vitro* effect of these hormones on D-2 DA receptors. We observe that *in vitro* E_2 or P alone do not affect D-2 DA receptors as measured by apomorphine competition of [^{3}H]spiperone binding. GTP produces a shift of the high to low affinity state of the D-2 DA receptors. When E_2 (1 nM) is included with GTP, the expected GTP-dependent shift of affinities is prevented. However, addition of Mg++, which is known to alter agonist binding properties of D-2 DA receptors, abolishes this E_2 effect. Although P shares common characteristics with E_2 on DA release activity, this steroid hormone does not alter agonist properties of D-2 DA receptors neither with an *in vivo* injection of P (100 µg) nor when added (100 nM) into *in vitro* competition experiments. These results suggest that the rapid increase of striatal DA release produced by E_2 could be mediated by an interaction with a G protein, which in turn affects the agonist states of the D-2 DA autoreceptors to ultimately induce DA release. P does not interact with this protein and seems to have an indirect action on DA release. Supported by the MRC of Canada.

479.7

EFFECTS OF NIGROSTRIATAL 6-HYDROXYDOPAMINE LESIONS ON DOPAMINE (D_2) RECEPTOR mRNA AND RECEPTOR BINDING. A.Mansour, J.H. Meador-Woodruff, D.M. Camp*, T.E. Robinson, J. Bunzow*, H. Van Tol*, O. Civelli*, H. Akil and S.J. Watson. Mental Health Research Institute and Neuroscience Building, University of Michigan, Ann Arbor, MI 48109-0720; The Oregon Health Sciences University, Portland, OR 97201.

The D_2 dopamine receptor has recently been cloned (Bunzow et al., Nature, 336:783-787, 1989) and its distribution has been described in the rat brain. These studies have provided a framework for examining the regulation of this receptor at both the level of its mRNA and binding site. Male Sprague-Dawley rats (N=11) were given a unilateral 6-hydroxydopamine (6-OHDA) lesion in the medial forebrain bundle (8 µg/4 µl, with 15 mg/kg DMI pretreatment) and sacrificed 16 days later. Such lesions produce a loss of dopaminergic cell bodies in the substantia nigra (SN) and ventral tegmental area (VTA) resulting in denervation of the striatum and an increase in DA receptor binding. The mechanism for this postsynaptic denervation supersensitivity is unclear. Preliminary *in situ* hybridization and receptor autoradiographic results suggest that these lesions produce a unilateral loss of D_2 receptor mRNA and receptor binding ([^{3}H] raclopride) in the SN and VTA and a concomitant increase in D_2 mRNA and binding in the striatum on the lesioned side. It appears, therefore, that D_2 receptors are localized on DA neurons of the SN and VTA where they regulate DA activity. Lesioning of these neurons produces an upregulation of postsynaptic D_2 receptor message, presumably increasing D_2 receptor expression. [Supported by DA02265, MH422251, MH45614, DK37231, Theophile Raphael Fund, and Lucille P. Markey Trust].

479.8

LOCALIZATION AND REGULATION OF BRAIN D_2-DOPAMINE RECEPTOR mRNA. J.H. Meador-Woodruff, A. Mansour, J.R. Bunzow*, H.H.M. Von Tol*, O. Civelli* and S.J. Watson. Mental Health Research Institute, University of Michigan, Ann Arbor, MI 48109-0720; Vollum Institute for Advanced Biomedical Research, The Oregon Health Sciences University, Portland, OR 97201.

The rat D_2 receptor has been recently cloned (Bunzow et al. Nature, 336:783-787, 1988), and we have previously mapped the distribution of its mRNA in rat brain. Highest levels of this mRNA have been localized in the caudate-putamen, nucleus accumbens, limbic cortex, and olfactory tubercle, although specific hybridization was identified in all traditional dopamine projection areas. In addition, high levels of D_2 receptor mRNA have been visualized in the substantia nigra, ventral tegmental area, and zona incerta, presumably reflecting autoreceptor synthesis. In our ongoing characterization of D_2 receptor mRNA in brain, we have examined changes in this mRNA following treatment with dopamine agonists and antagonists. Male Sprague-Dawley rats were treated with chronic haloperidol (14 days, 2 mg/kg/day) or apomorphine (7 days, 5 mg/kg/12 hrs). Following these treatments, brains were removed, frozen, and sectioned. 15 um sections were examined by *in situ* hybridization using ^{35}S-labelled riboprobes complementary to mRNA coding for the putative sixth and seventh transmembrane domains and the G-protein-associated third cytosolic loop of this receptor. Results of these analyses in both traditional dopamine projection fields and in areas associated with dopamine-containing cell bodies will be presented. This work supported by Grants DA02265, MH422251, MH45614, and funds from The Theophile Raphael Fund and The Lucille P. Markey Charitable Trust.

479.9

HALOPERIDOL-INDUCED DOPAMINE (D_2) RECEPTOR UP-REGULATION: EFFECT OF CHRONIC COADMINISTRATION OF RITANSERIN. **C.A. Wilmot, A.M. Szczepanik and D.B. Ellis*** Dept. Biol. Res., Hoechst-Roussel Pharmaceuticals Inc., Somerville, NJ 08876-1258.

The chronic administration of neuroleptics produces an up-regulation of D_2 receptors which may contribute to the adverse motor side effects of these compounds. Chronic treatment with clozapine, an atypical antipsychotic with a low incidence of dyskinesias, does not affect D_2 receptor number. Clozapine is a potent serotonin ($5HT_2$) antagonist which produces a significant down-regulation of $5HT_2$ receptors. The objective of the present study was to determine whether the chronic coadministration of the $5HT_2$ antagonist ritanserin (RIT) affects the D_2 receptor up-regulation produced by haloperidol (HAL). Rats were treated for 21 days with RIT (5 mg/kg, ip), HAL (1 mg/kg, ip), both RIT and HAL or vehicle and killed 3 days following the last dose. Brain sections were prepared for D_2 and $5HT_2$ receptor autoradiography with [^{3}H]spiperone and [^{3}H]ketanserin. Striatal and n. accumbens D_2 receptors were significantly increased in HAL-treated rats, with the greatest up-regulation in the ventrolateral striatum. Chronic RIT alone decreased the number of cortical $5HT_2$ receptors but had no effect on D_2 receptors. In RIT/HAL-treated rats, D_2 receptors were up-regulated together with a significant decrease in the number of $5HT_2$ receptors. These results suggest that the down-regulation of $5HT_2$ receptors produced by chronic treatment with $5HT_2$ antagonists does not affect neuroleptic-induced D_2 receptor up-regulation.

479.10

NEONATAL 6-OHDA DENERVATION ELEVATES DOPAMINE D_2 BUT NOT D_1 RECEPTORS IN ADULT RAT NEOSTRIATUM. K.M. Dewar, J.-J. Soghomonian, J. Bruno, L. Descarries and T.A. Reader. CRSN, Dép. de physiologie, Univ. de Montréal, Montréal, (Qué.) Canada and Department of Psychology, Ohio State University, Columbus, Ohio, U.S.A.

The binding properties of [^{3}H]SCH23390 and [^{3}H]raclopride were investigated to evaluate D_1 and D_2 dopamine (DA) receptors in homogenates of rostral (rCAU) and caudal (cCAU) neostriatum at 1 and 3 months after the neonatal i.c.v. administration of 6-OHDA in the presence of DMI (to protect the noradrenergic system). HPLC-ED measurements indicated that DA levels were decreased by > 90% in the rCAU and cCAU at both time intervals. In addition, 5-HT and 5-HIAA concentrations were considerably augmented in the rCAU at 1 and 3 months. There were no changes of D_1 receptor binding in the rCAU and cCAU at either time period. In contrast, the density of D_2 sites was significantly increased in the rCAU but not in the cCAU. These results indicate that, in the neostriatum, D_2 and not D_1 receptors are modified by a neonatal DA denervation. Furthermore, since the up-regulation of [^{3}H]raclopride sites is restricted to the rCAU which also exhibits a 5-HT hyperinnervation, the data suggest that these receptors are located on the 5-HT fibers and/or regulated by the 5-HT system.

[Supported by the MRC (Canada) and the FRSQ (Québec).]

479.11

PHORBOL ESTERS INHIBIT MUSCARINIC RECEPTOR DOWN-REGULATION IN 1321N1 HUMAN ASTROCYTOMA CELLS. M.L. Toews* and R.K. Hoover* (SPON:J. Firman) Dept. of Pharmacology, Univ. of Missouri, Columbia, MO 65212.

Binding of ^{3}H-QNB to intact cells was used to assess the effects of phorbol esters on down-regulation of muscarinic receptors (MR) in 1321N1 human astrocytoma cells. The agonist carbachol induced down-regulation of MR to about 20% of control levels, with a half-time of about 2 hrs. Phorbol 12-myristate, 13-acetate (PMA) alone did not induce MR downregulation. Inclusion of PMA during carbachol-induced down-regulation inhibited both the rate and maximal extent of receptor down-regulation. The protein kinase C (PKC) activators mezerein, phorbol dibutyrate, and ß-phorbol didecanoate also inhibited MR down-regulation, whereas the inactive analog α-phorbol didecanoate was ineffective. The PKC inhibitor staurosporine did not inhibit carbachol-induced down-regulation but did prevent the inhibitory effect of PMA. The effect of PMA on down-regulation was observed in the presence of cycloheximide, suggesting that activation of PKC inhibits loss of MR rather than stimulating MR synthesis. (Supported by GM34500 and HL01593).

479.12

RECIPROCAL INTERACTION BETWEEN M-RECEPTOR AND PROTEIN KINASE C IN CULTURED NEURONS FROM HIPPOCAMPUS. V. Alemán, B. Osorio* and J.L. Camacho*. Dept. of Physiology. CINVESTAV-IPN, México City, México 07000.

Ten day-old rats from both sexes were decapitated hippocampi dissected and their neurons dissociated and cultured during 10 days. Neurons were then treated during 24 hr. adding either different amounts of carbachol (0,2.5,10,20, 50,100,750 and 1,500 µM) or different concentrations of 12-0-tetradecanoylphorbol 13-acetate (TPA): 0,10,20,30,40,80 and 200 nM. After this time, cells were harvested and P_2 fraction obtained. Determination of muscarinic receptor was carried out using 40 µg of protein sample and 10 nM [N-methyl-^{3}H] scopolamine, nonspecific binding was determined in presence of 10 µM atropine. For the determination of [^{3}H] Phorbol-12-13 dibutyrate ([^{3}H] PDBu) binding sites, a P_2protein sample of 300 µg in presence of 60 nM concentration of [^{3}H] PDBu was used. In these studies we found a relationship between increasing concentrations of carbachol and decreasing muscarinic receptors number. Opposite effects were obtained with the amount of [^{3}H] PDBu binding sites at the same concentrations of carbachol. On the other hand when cultures were incubated in presence of increasing concentrations of TPA, the value of scopolamine binding sites increased significatively. We don't understand why the muscarinic binding sites increased in presence of TPA however, this finding suggest a functional compling in both directions between muscarinic receptors and protein kinase C.

479.13

MUSCARINIC AND NICOTINIC RECEPTORS AND THEIR mRNAS ARE REGULATED DIFFERENTLY IN CULTURED SYMPATHETIC NEURONS. **K.E. Smith, V.Wong, and J.A. Kessler, Departments of Neurology and Neuroscience, Albert Einstein College of Medicine, Bronx, New York, 10461.**

Sympathetic neurons cultured from the neonatal rat superior cervical ganglion (SCG) express both muscarinic and nicotinic cholinergic receptors. We have been examining the regulation of cholinergic receptors by signals in the neuronal microenvironment which have been shown to affect neurotransmitter expression. For example, a membrane-associated factor MANS (Wong and Kessler, 1987) which stimulates activity of choline acetyltransferase (ChaT) in SCG neurons caused a large reduction in the number of muscarinic receptors and a corresponding decrease in muscarinic M2 mRNA levels. By contrast, no change was observed in levels of nicotinic receptors or in levels of nicotinic receptor α3 or ß2 subunit mRNAs. Similarly, a soluble factor produced by rat fibroblasts (RFCM) which also stimulated ChAT activity and decreased muscarinic receptor number in SCG neurons had no effect on levels of nicotinic receptors or their mRNAs. These data suggest that nicotinic receptor function may not be regulated by changes in receptor number, and that regulatory mechanisms for muscarinic and nicotinic receptors in the SCG are fundamentally different. We are currently examining the regulation of ß2-adrenergic receptors and receptor mRNA levels by similar microenvironmental signals.

479.14

ACCELERATION OF CENTRAL MUSCARINIC RECEPTOR TOLERANCE BY INDOMETHACIN. A.C. Hays*, M.H. Sullivan*, W.C. Hallowes* and J.J. Buccafusco (SPON: G.O. Carrier). Dept. Pharmacology and Toxicology, Medical College of Georgia and Veterans Administration Medical Center, Augusta, GA 30912.

Our previous studies have demonstrated a significant tolerance to the hypertensive response to central (cerebroventricular) injection of carbachol (CARB) in conscious rats. This pressor response exhibits tachyphylaxis if the injection is repeated in less than 8 hr after the first injection. Blockade of brain prostaglandin synthesis with indomethacin (INDO) does not inhibit the pressor response to CARB in naive rats, but eliminates the pressor response to CARB when the CARB is repeated within a few hr after the first injection. If the time interval is extended so as to allow for the complete return of the full response (i.e., 24hr later), INDO no longer inhibits. When shorter acting drugs (duration of action < 30 min), physostigmine or arecoline, were employed using the same paradigm, INDO was not affective in inhibiting the pressor response to the second injection, even when the two agonist injections were spaced only 30 min apart. However, if CARB was used for the first injection, INDO blocked the response to subsequent (2hr) injection of physostigmine. This ability of INDO to enhance central muscarinic receptor tolerance was extended to CARB-induced hypothermia. Prostaglandins may play a role in the mechanism of development of tolerance to muscarinic receptor stimulation. Thus, activation of prostaglandin synthesis, in vivo, may function to decelerate the development of tolerance to muscarinic agonists. Supported by: NIH, HL&B and the Veterans Administration.

479.15

REGULATION OF MUSCARINIC ACH, ADENOSINE AND NICOTINE RECEPTORS AND THE ROLE OF INTRACELLULAR MESSENGERS F. van Huizen, C. Shaw and M. Cynader, Dept. of Ophthalmology, Univ. British Columbia, Vancouver, B.C., Canada V5Z 1V5

Muscarinic ACh receptors (mAChRs) in cerebral cortex slices are downregulated by increasing neural activity with veratridine or by activating the receptors with carbachol (*Mol.Brain Res. 5, 59-69 & 71-83*). The effect can be blocked by the K^+-channel blockers TEA and apamin. To investigate the role of intracellular messengers (IM) in receptor downregulation, slices were incubated for 4h at 37°C with a wide variety of drugs acting on IM before being incubated for 2h at 4 °C with [3H]NMS to label surface mAChRs. Forskolin and choleratoxin (activators of adenylate cyclase), NEM (inactivator of G-proteins) and arachidonic acid (activator of pertussis toxin sensitive G-proteins) had no effect on mAChR number. Of the GTP analogues Gpp(NH)p, GDPßs and GTPγs, only the latter had a slight effect on mAChR number (-6.9%), which could be blocked by simultaneously adding TEA. Apparently, mAChR downregulation is modulated by the IP3 system and not the cAMP system, since stimulating PKC with phorbol esters is known to downregulate mAChRs (*Jia et al., Mol. Brain Res., 1989*). Adenosine receptors ([3H]CPDX) were downregulated after preincubation with a mixture of glutamic acid and veratridine (Glu/Ver; -12.5%) and after agonist stimulation with CHA (-65%). This effect was not due to displacement and was partially blocked by TEA (-55.3%). GTPγs had no effect. Nicotine receptors ([3H]Nicotine) were also regulated by increased neural activity (Glu/Ver: -36.1%) and by receptor stimulation (Nicotine: -69.2%).

479.16

ESTRADIOL AND PROGESTERONE EFFECTS ON MUSCARINIC ACETYLCHOLINE RECEPTORS IN RAT CERBRAL CORTEX

D. F. March*, F. van Huizen, C. Shaw (Spon. J. Steeves) Dept. of Ophthalmology, U.B.C., Vancouver, B.C., Canada.

Past studies have indicated that muscarinic acetylcholine receptors (mAChRs) are regulated by Estradiol (E_2) and Progesterone (P) in specific areas of the rat brain. In this study, the effects of E_2 and P on mAChRs in female rat cerebral cortex were investigated to examine the relationship between the onset of puberty and the peak of mAChR binding on day 30-32 postnatal for the rat cerebral cortex.

Groups of 6-8 female littermates of similiar weight (50 gms) were ovariectomized at 22 days of age. At 29 days of age the animals were sacrificed. Using an in vitro "living" brain slice assay (van Huizen et. al.(1989) Mol. Brain Res. 5:59-69), 400um thick cortical slices were preincubated for 90 minutes at 37°C with: E_2 (10^{-7}M), P (10^{-6}M), E_2 and P, or control (C). The four groups were then incubated for 2 hours at 4°C with [^{3}H]-N-methyl-scopolamine in increasing concentrations (0.15nM-40nM) to obtain saturation binding curves. The present results suggest that ovariectomy increases mAChR number in cortex over normal values. The addition of the gonadal hormone E_2 in vitro, decreases mAChR Bmax by 20% from ovariectomy alone; P addition alone shows a similiar decrease. The addition of both E_2 and P decreases binding by a further 10%. The Kd values for E_2 are also decreased when compared to ovariectomy (27%), P alone decreased the Kd only slightly less than E_2 while E_2 and P decrease more than E_2.

479.17

CHRONIC ADMINISTRATION OF NICOTINIC ANALOGS: TOLERANCE AND RECEPTOR REGULATION. R. V. Bhat*; S. L. Turner*; M. J. Marks* and A. C. Collins. Sch. of Pharm. and Inst. for Behav. Genetics; Univ. of Colorado, Boulder, CO 80309.

Chronic nicotine treatment results in tolerance development and unlike normal agonist treatment upregulates the nicotinic cholinergic receptors (nChR). This upregulation could be specific to the agonist nicotine itself, or a property of the nChR. Consequently, two other nicotinic analogs, anabasine and lobeline, were tested for their chronic effects on tolerance development and receptor regulation. Equimolar concentrations of anabasine, lobeline, and nicotine or saline were continuously infused into C57BL/6 mice for nine days. Tolerance tests (respiration, Y-maze crosses and rears, startle response, heart rate and body temperature) were then conducted following a challenge dose of the infused drug. Tolerance was observed in the nicotine treated mice but not in the other treatment groups. Cross-tolerance was examined and results indicate a lack of cross-tolerance between any of the nicotinic analogs. Microdissection of the brains and receptor assays indicated an upregulation of nChR in all drug treatment groups but no changes in muscarinic receptors was seen. This study furthers the notion that upregulation of the nChR following chronic agonist treatment is a property of the receptor itself and is not just specific to nicotine. **Supported by DA-03194 and DA-00116.**

479.18

THE THYMIC POLYPEPTIDE THYMOPOIETIN REGULATES α-BUNGAROTOXIN RECEPTORS IN CHROMAFFIN CELLS IN CULTURE. J. Philie*, R. Afar*, J.M. Trifaro*, T. Audhya*, G. Goldstein* and M. Quik. Depts. Pharmacol., McGill U. & U. Ottawa, Canada & Immunobiol. Res. Inst. Annanadale, NJ

Recent work in our laboratory showed that the thymic polypeptide thymopoietin potently and specifically interacts at the nicotinic α-bungarotoxin (α-BGT) site in brain. To gain insight into its possible role in nervous tissue the effect of thymopoietin was studied in cultured chromaffin cells. To determine whether thymopoietin interacted directly at the α-BGT site in chromaffin cells, cells in culture were preincubated for 60 min with thymopoietin and ^{125}I-α-BGT determined; a dose dependent inhibition was observed. Incubation of the cells in culture with thymopoietin (10^{-8} to 10^{-6} M) on a long term basis (3 to 6 d), resulted in a 2-3 fold increase in α-BGT binding. The thymopoietin-induced increase in binding could be reversed with nicotine; thus, the sites can be regulated by a nicotinic receptor ligand. Although thymopoietin potently interacted at the nicotinic α-BGT receptor, the peptide did not affect nicotinic sensitivity; neither basal nor nicotinic receptor stimulated tyrosine hydroxylase activity was altered by thymopoietin. These results indicate that the thymic polypeptide thymopoietin may act as a specific regulator of the nicotinic α-BGT site in chromaffin cells.

479.19

MELATONIN INHIBITS ^{35}S-TBPS BINDING IN RAT BRAIN. L.P. Niles. Dept. Biomedical Sciences, McMaster University, 1200 Main Street West, Hamilton, Ontario, L8N 3Z5.

In order to further clarify melatonin's interaction with the GABA-benzodiazephine (BZ) receptor complex, its effects on the binding characteristics of the cage convulsant, [^{35}S]t-butylbicyclophosphorothionate ([^{35}S]TBPS), which binds to recognition sites on or associated with GABA-gated chloride channels were examined.

Binding assays were carried out with fresh or frozen rat brain membranes. Non-specific binding was measured in the presence of 20 μM picrotoxin. In fresh unwashed forebrain membranes, melatonin inhibited binding by up to 56% with an IC_{50} of ~250 μM. In washed membranes, binding was maximally inhibited by 75% and melatonin had an IC_{50} of ~ 200 μM. A comparison of melatonin with various tryptamines and catecholamines indicated that only melatonin consistently inhibited [^{35}S]TBPS binding.

Saturation binding experiments conducted with or without melatonin (10 or 100 μM) indicated that its effect is due to a decrease in the density of TBPS binding sites with a concomitant increase in binding affinity. These findings indicate that melatonin allosterically inhibits TBPS binding as recently reported for GABA-positive BZ's, like diazepam. (Supported by the OMHF and MRC Canada.)

479.20

CLASSIFICATION OF DRUGS AS ANTAGONISTS OR AGONISTS AT $A_{1,3}$ADENOSINE RECEPTORS BY MEASURING INHIBITION OF [^{3}H]CYCLOHEXYLADENOSINE BINDING. S.M. Anderson, J.W. Stauffer*; R.L. Weir* and J.W. Daly. Dept of Medical Neurosciences, Walter Reed Army Institute of Research, Washington, DC 20307; Neurotoxicology Section, NINCDS, NIH, Bethesda, MD 20892, and Dept of Neurology, Howard U School of Medicine, Washington, D.C. 20060; Lab of Bioorganic Chemistry, NIDDKD, NIH, Bethesda, MD 20892

Agonists for adenylate cyclase-linked receptors have low and high affinity binding states. Antagonists, in contrast, bind to only one state of these receptors. Data from saturation binding of [^{3}H]CHA to cortical membranes from rat brain are described best by the presence of two agonist binding states. Using data from drug inhibition experiments, we estimated Ki_h's at the higher affinity state by using the approximation that all binding at 0.1 nM [^{3}H]CHA is to the higher affinity state. The high affinity binding component was subtracted from the amount of 10 nM [^{3}H]CHA bound, where there was substantial [^{3}H]CHA binding at both the high and lower affinity states, and Ki_l's for the lower state estimated. Using this protocol, agonists used to inhibit [^{3}H]CHA binding were characterized by different Ki's, Ki_h = 0.64 nM and 6.7 nM, Ki_l = 11 nM and 132 nM, for R-PIA and S-PIA. However, Ki's for antagonists' inhibition of [^{3}H]CHA binding to the higher and lower affinity states are equivalent, Ki_h = 7.0 uM and 36 uM, Ki_l = 7.2 uM and 37 uM, for theophylline and caffeine. This protocol is useful for defining A_1 adenosine receptor agonist/antagonist properties of new compounds.

BLOOD-BRAIN BARRIER III

480.1

HOST AND DONOR TISSUE CONTRIBUTE TO THE BBB FOLLOWING BRAIN GRAFTING N.Akalan*, J.Leonard*, M.S.Grady (SPON:D.Sutton) Dept. of Neurosurgery, Univ. of Washington, Seattle, WA 98040.

Solid and suspension grafts of fetal CNS tissue rapidly reform an intact BBB whereas solid grafts of PNS tissue fail to reform a BBB as detected by the leakage of HRP. We examined the acute changes in the BBB following grafting of fetal CNS tissue in a solid and cell suspension form and PNS tissue such as superior cervical ganglion (SCG) in the same manner.

Adult rats(n=20) received fetal (E14-15) forebrain grafts (either solid or cell suspension) in the rostral corpus callosum bilaterally. The second group(n=20) received adult SCG solid and cell suspension grafts at the same coordinates with the same technique. The animals were killed on the first, third, seventh, and tenth days following grafting. Intravenous HRP Sigma type VI, 75 mg/5 gm rat was given one hour prior to perfusion with mixed aldehydes. Fifty micron coronal serial sections were examined for the presence and location of graft(cresyl violet and AChE) and the Mesulam's TMB method was performed for HRP leakage.

HRP leakage was detected in the surrounding parenchyma in all groups on the first and third days post-transplantation. No HRP reaction was seen at the seventh and tenth day post-transplantation in groups receiving fetal forebrain tissue whether solid or suspension. Solid grafts of SCG consistently demonstrated HRP leakage from the first through the tenth day. However, cell suspension of SCG formed a BBB by seven days. These results suggest that the vascular supply of the grafted tissue expresses BBB properties dictated by both the grafted tissue and the neural environment surrounding the graft.

480.2

ULTRASTRUCTURAL AND QUANTITATIVE STUDIES OF THE BLOOD-BRAIN BARRIER IN INFLAMMATION. L. Claudio*, J. Martiney*, Y. Kress* and C. Brosnan*. (SPON: RD Ledeen) Dept of Pathology, Albert Einstein College of Medicine, Bronx, New York 10461

Mechanisms for loss of blood-brain barrier (BBB) function were studied in experimental autoimmune encephalomyelitis in the Lewis rat and in the rabbit visual system. In EAE horseradish peroxidase (HRP) was used as an intravascular tracer. Quantitative morphometry of endocytic endothelial cell vesicles demonstrated a 5.2-fold increase over the normal values at the peak of clinical index, which then resided to twice the normal levels during the recovery period. A concomitant decrease in endothelial mitochondrial area was observed. Electron micrographs showed the localization of HRP in pinocytic vesicles, lysosomes and cannalicular structures. Rats perfusion-fixed prior to HRP infusion showed tracer leakage through the tight junction in areas of extensive inflammation. In order to study the direct effects of cytokines on endothelial cells, intraocular injection into the rabbit was used. The results showed that the cytokines IL-1, IL-2, interferon-gamma and tumor necrosis factor induced changes in blood-retina barrier morphology, similar to those seen in endothelia cells in EAE. The data presented suggests that inflammatory cells can induce BBB changes through the release of cytokines.

480.3

IMMUNOCYTOCHEMICAL LOCALIZATION OF A MULTIDRUG-RESISTANT GLYCOPROTEIN IN CNS VASCULATURE OF THE RAT. J.A. Ellison*, J.J. Hall* and L.J. Noble. Dept.of Neurology, University of California, VAMC, San Francisco, CA 94121.

Multidrug-resistance refers to the ability of a cell to exclude certain cytotoxic products, and it has been postulated that failure of chemotherapy is in part attributed to the development of drug resistance in cancer cells to multiple pharmacologic regimens. Multidrug resistant cancer cells are characterized by a unique glycoprotein (P-GP) which is hypothesized to act as a pump to remove toxic agents from cells.

Using a monoclonal antibody specific for P-GP (Centocor, Malvern, PA), we have evaluated the immunocytochemical distribution of this glycoprotein in the spinal cord of the rat. Immunoreactivity was observed in a number of cell types including glia, which appeared darkly stained, the apical portion of ependymal cells, and blood vessels. With regard to the latter, not all vessels were immunoreactive. In white matter, fewer vascular segments were immunostained (as compared to grey matter) and immunostaining was not uniform along a given vascular length. In grey matter, partial staining was observed in a majority of the capillaries. The association of P-GP with normal CNS vasculature suggests that the glycoprotein may provide an additional line of defense at the level of the blood-brain barrier. Supported by NS23324 to LJN.

480.4

IMMUNOLOGIC CHARACTERIZATION OF A SYSTEMIC DRUG RESCUE MODEL BASED ON BLOOD-BRAIN BARRIER DIFFERENTIAL PERMEABILITY. J.M. Nazzaro, L.C. Rosenbaum* & E.A. Neuwelt*. Depts. of Biochem. and Surgery (Div of Neurosurgery), Oregon Health Sciences University, Portland, OR.

Previous studies have suggested drug antibody (Ab) can bind systemic drug without significantly affecting free therapeutic drug delivery to brain lesions. We now report on immunologic characterization to further define specificity of the drug-Ab binding. Brain abscess-bearing rats received [125I] gentamicin (GTM; MW 462) IV followed by specific rabbit antiserum (IgG; MW 150,000) to GTM (immune) or normal rabbit serum (nonimmune). Serum equilibration with biotinylated antirat IgG followed by avidin-agarose failed to show significant GTM binding (<1%) suggesting no interaction with endogenous IgG. Incubation of serum supernates with protein A-Sepharose (pA-S) showed significant immunobound GTM only in immune animals ($p<0.001$). Homogenates of lesions showed similar percentages (25%) of nonspecifically brain-bound drug in both groups. Incubation of brain supernates with pA-S resulted in no appreciable GTM binding suggesting significant IgG exclusion from the lesion. Our results indicate that drug Ab can be used to bind specifically a high percentage of systemic drug and support further research of a drug rescue method based on BBB differential permeability to low- and high-molecular-weight compounds.

480.5

DEVELOPMENTAL CHANGES IN ENDONEURIAL ALBUMIN DYNAMICS IN RAT SCIATIC NERVE. J. Poduslo, A. Weerasuriya*, and G. Curran*. Peripheral Nerve Center, Mayo Clinic and Foundation, Rochester, MN 55905

The rate of entry of albumin into the endoneurial space and its fate within that compartment were investigated by measuring the permeability coefficient-surface area product (PS) of the blood-nerve interface (BNI) to ^{125}I-albumin, residual endoneurial plasma volume (V_p), and the BNI index to albumin in sciatic nerves of 1, 2, 3, 4, 6, 8, and 13 week old rats. The results are given as mean ± SEM.

Age (Weeks)	PS (ml/g/s)x10^6	V_p (µl/g)	Alb-BNI Index(%)	Wet/Dry Weight
1			3.6±0.1	4.48±0.07
2	6.8±0.3	3.9±0.5	7.6±0.5	3.41±0.08
3	3.6±0.4	4.0±0.5	7.0±0.6	3.29±0.04
4	2.0±0.2	2.2±0.1	10.3±1.7	3.29±0.04
6	1.0±0.2	2.6±0.3	13.4±0.8	3.26±0.02
8	0.7±0.1	1.5±0.2	13.1±0.8	3.13±0.02
13	0.7±0.1	1.4±0.1	5.0±0.2	3.06±0.02

A tenfold larger PS at two weeks, compared to 13 weeks, is due both to a larger P, as well as S, in the infant rat. The smaller Alb-BNI index at 1, 2, and 3 weeks indicates that albumin is probably cleared from the endoneurium by the epi- and perineurial lymphatics. Subsequently, as the perineurium becomes less permeable, there is a transient increase albumin in the endoneurium, and this decreases towards adult values as both components of BNI become less permeable. (NS14304-P4 and Borchard Fund).

480.6

CHANGES IN ENDONEURIAL BLOOD FLOW IN RAT SCIATIC NERVE DURING DEVELOPMENT. M. Kihara*, A. Weerasuriya*, and P.A. Low (SPON: C. Kornblith). Department of Neurology, Mayo Foundation, Rochester, MN 55905.

To investigate the mechanism by which the developing nerve with a low mean arterial pressure (MAP) maintains adequate vascular perfusion to meet the demands of enhanced oxygen consumption and metabolic rate, nerve blood flow (NBF) was measured in sciatic nerve of 2, 3, 4, 6, 8, and 12-week-old rats with the microelectrode H_2 clearance technique. The results are given as mean ± SEM.

Age (weeks)	n	NBF (ml/100 g/min)	MAP (mm Hg)	VR (NBF/MAP)
2	6	28.8±2.4	54±4	1.9±0.2
3	6	29.2±0.6	82±12	2.8±0.4
4	7	20.6±1.6	100±7	5.1±0.5
6	6	23.0±2.0	130±7	6.0±0.7
8	6	16.1±2.2	142±7	9.8±1.7
12	6	14.1±1.9	131±5	10.1±1.3

NBF, MAP, and vascular resistance (VR) reach adult values by 8-12 weeks of age. NBF and VR of 12-week sciatic, tibial, and sural nerves are not significantly different. The last two are comparable in diameter to 2-3 week sciatic nerve. It is likely that the higher endoneurial vascular volume in young rats contributes to the lower vascular resistance but other factors need to be identified and characterized for a complete description of hemodynamics in the immature endoneurium. (NS14304, NS22352, and Borchard Fund).

480.7

MIANSERIN ALTERS CEREBROMICROCIRCULATORY RESPONSIVITY TO CHANGES IN ARTERIAL CO_2. Steve J. Bupp, M.D., Sheldon H. Preskorn, M.D., Julie Schwartzman, M.D. -VAMC, Univ. of Kansas, Psychiatric Research Institute at St. Francis, Wichita Ks. 67214

Tricyclic antidepressants potentiate the $PaCO_2$-induced increase in cerebral capillary permeability to a diffusion-limited substance(PS) via their effects on central adrenergic neurons. Mianserin is an antidepressant which acts as a direct alpha$_2$ and alpha$_1$ agonist without reuptake blockade properties. We studied its ability to alter adrenergic regulation of PS and cerebral blood flow (CBF).

Sprague-Dawley rats were divided into 6 groups: control, and mianserin doses of 1, 10, 100, 1000 ug/kg i.v., and 1 ug/kg i.c.v.. Rats in each group were passively ventilated with O_2, NO_2 and varying amounts of CO_2. A dual label isotope procedure (Irwin and Preskorn, Brain Res., 1982) was used to simultaneously measure CBF and PS to water in five brain regions.

Mianserin did not affect arterial blood gases or mean arterial pressure at any dose, but it did diminished the normal tight coupling of CBF to $PaCO_2$ at the 100, and 1000ug/kg dose. Mianserin caused significantly higher values of PS at a $PaCO_2$ of 20 mm Hg. compared to control animals in all brain regions in a dose dependent fashion; however, the functional relationship between PS and $PaCO_2$ was not different from controls at higher $PaCO_2$'s (80 mm Hg.). Thus, Mianserin, like other antidepressants, significantly disrupts the normal cerebromicrocirculatory regulation.

480.8

Amitriptyline Effect on Brain Fluid Dynamics. S. Preskorn, I. Watanabe, A. Dick, S. Henson, (Spon: C. Hughes) VAMC, the U. of Kansas, and Psychiatric Research Institute, Wichita, KS.

Tricyclic antidepressants (TCAs) increase blood brain barrier (BBB) permeability to water (PS) and affect volume and composition of brain extracellular fluid compartment via adrenergic mechanisms. This study evaluated changes in brain ultrastructure and water content coincident with PS changes. Amitriptyline (AMI), 15 and 30 mg/kg i.p. was given to Sprague-Dawley rats under acute (15 min. before sacrifice, n=6) and chronic (daily x 10 days, n=6) conditions. Half of the chronically treated animals received an acute injection. The anesthetized and heparinized animals were sacrificed by intra-cardiac perfusion fixation. Tissue was processed for light and electron microscopy. The same design tested AMI effect on brain specific gravity, measured by the gradient column method. There were controls for both projects. Electron microscopy revealed swelling of endothelial cells with increased pinocytic activity and of fibrillary astrocytes particularly their perivascular foot process. There was no disruption of the BBB anatomical integrity and no neuronal changes. There was no regional changes in brain specific gravity. Thus PS changes are associated with physiologically significant but reversible changes in fluid dynamics and could underlie ability of norepinephrine to modulate the response of neurons in remote areas to afferent input.

480.9

RAPID SEALING OF BLOOD-BRAIN BARRIER LEAKS AFTER ETHANOL PLUS BARBITURATE-INDUCED DAMAGE. P.A. Stewart, J.A. Holash*, C.R. Farrell* and E.M. Hayakawa*. Dept. of Anatomy, Univ. of Toronto. Toronto, Ontario, Canada. M5S 1A8

Ethanol in high doses induces multifocal leaks in the blood-brain barrier (BBB) by lysing a population of brain endothelial cells. Administration of thiopental, a commonly used anesthetic agent in humans, acts synergistically with ethanol to cause similar barrier damage at much lower doses of ethanol. The time course of barrier breakdown and recovery was investigated using horseradish peroxidase (HRP) - a vascular tracer that does not cross the intact BBB. Tissue levels of HRP were measured in cerebral cortex, hippocampus and cerebellum. HRP was significantly elevated in all three areas 5 minutes after drug administration, and remained high for at least one hour. By two hours total HRP had decreased to control levels, where it remained at least to six hours. This rapid breakdown of the barrier is consistent with gross structural damage to endothelial cells seen previously. The rapid rate of sealing the barrier leaks suggest that fibrin clot formation initiated by endothelial lysis may be responsible. Supported by MRC Canada.

480.10

Alteration of blood-brain barrier permeability in a bacterial cerebritis. Warren D. Lo and David McNeely*. Dept. of Pediatrics, Ohio State Univ., Columbus, OH

Inoculation of Staph. aureus (S. aureus) into the rat brain induces an influx of neutrophils into the brain within 4-6 hours. We have reported that in this model abnormal cerebrovascular permeability, as assessed by leakage of Evans Blue, does not develop until day 3 in the S.aureus inoculated brain (Soc. Neurosci. 13:493, 1987).

We measured gray matter specific gravity as an indication of tissue water content. On day 4 the specific gravity of the S. aureus-inoculated cortex was 1.0465+/-0.0026 (n=11) vs. 1.0485+/-0.0007 (n=11) in control cortex ($p<0.05$); thus water accumulated in the inoculated cortex. We used ^{14}C-sucrose to measure the brain permeability-surface area product (PS) in anesthetized rats. The PS of the inoculated region (in mls/gm-min x 10-3) on day 0 was 1.99+/-0.40 (n=4) in experimental animals vs. 2.32+/-0.63 (n=4) in controls (not significant (N.S.)); on day 4 these values were 2.41+/-0.56 (n=7) (experimental animals) vs. 2.44+/-1.34 (n=6) (controls) (N.S.). Thus, when Evans Blue leakage demonstrates increased blood-brain barrier permeability, there is no net movement of sucrose into the brain. We speculate that in a cerebritis, the nature of an intravascular tracer (i.e. polarity or charge) determines its movement into and retention by the brain.

480.11

BLOOD-BRAIN BARRIER (BBB) TRANSPORT OF CATIONIZED IMMUNOGLOBULIN G: ENHANCED DELIVERY COMPARED TO NATIVE PROTEIN. D. Triguero*, J.P. Buciak*, J. Yang*, and W.M. Pardridge (SPON: E. Cornford). Department of Medicine and Brain Research Institute, UCLA School of Medicine, Los Angeles, CA 90024.

Immunoglobulin G (IgG) molecules are potential neuropharmaceuticals that may be used for therapeutic or diagnostic purposes. The possibility of enhanced IgG delivery through the BBB by cationization of the proteins was explored in the present studies. Bovine native IgG molecules were cationized by the covalent coupling of hexamethylenediamine, and the isoelectric point was raised to >10.7 based on isoelectric focusing studies. Native or cationized IgG molecules were radiolabeled with [^{125}I]-iodine and chloramine T. Cationized IgG, but not native IgG, was rapidly taken up by isolated bovine brain microvessels used as an in vitro model system of the BBB. Cationized IgG binding was time- and temperature-dependent, and was saturated by increasing concentrations of unlabeled cationized IgG; K_D = 0.90 ± 0.37 μM, Bmax = 1.4 ± 0.4 nmol/mg$_p$. In vivo uptake of [^{125}I]-cationized IgG into brain parenchyma via transport through the BBB was documented by thaw-mount autoradiography of frozen sections of rat brain obtained following carotid arterial infusions of labeled protein. Conclusions: These studies demonstrate that cationization of IgG molecules greatly facilitates the transport of these plasma proteins through the BBB in vivo and that this process may provide a novel new strategy for IgG delivery through the BBB.

480.12

HUMAN BLOOD-BRAIN BARRIER DR-ANTIGEN. J. Yang*, W.M. Pardridge, J. Buciak*, and W.W. Tourtellotte* (SPON: C. Markham). Departments of Medicine and Neurology, UCLA School of Medicine, Los Angeles, CA 90024-1682.

Antigen presentation within the human central nervous system (CNS) by the class II histocompatibility or DR-antigen may take place at either the brain capillary-endothelial interface, which makes up the blood-brain barrier (BBB) in vivo, or at perivascular cells such as smooth muscle or pericytes. The microvascular DR-antigen was localized by avidin-biotin-immunoperoxidase studies using a mouse monoclonal antibody to the human DR-antigen or a mouse myeloma IgG2a control, and microvessels isolated from either fresh or frozen autopsied human brain. The DR-antigen was readily detectable in precapillary arteriolar smooth muscle cells in all subjects, but with increased deposition of DR-antigen immunoreactivity in the pericytes of capillaries isolated from multiple sclerosis brain. The capillary plasma membrain fraction was iodinated and the DR-antigen antibody precipitated a labeled 33K protein, which is identical to the molecular weight of the α-subunit of the human DR-antigen. Conclusions: These experiments show that the DR-antigen is readily detectable in human microvasculature of normal brain and is found in the smooth muscle cells of precapillary arterioles and in capillary pericytes with minimal, if any, staining of capillary endothelium. These results are consistent with the hypothesis that antigen presentation in the CNS occurs primarily at a site immediately distal to the blood-endothelial interface.

NEURAL PLASTICITY IN ADULT ANIMALS: SENSORY SYSTEMS

481.1

PLASTICITY OF VISCERAL AND SOMATIC AFFERENT SYSTEMS AFTER BRAIN DAMAGE; M.S.Sinyaya* and V.L.Silakov*. Pavlov Institute of Physiology of the Academy of Sci. of the USSR, 199034, Leningrad, USSR.

Plastic compensatory reorganization within somatic and visceral afferent systems is found in first somatosensory area of the cat cortex. We studied the reaction of cortical neurons on anaesthetized by chloralose and immobilized by flaxedil cats which survived an one-sided dissection of the projection cortical fibers two years before. As the compensatory of visceral and skin sensitivity developed after dissection, the neurons with monolateral sensitivity in the non-operated hemisphere converted into neurons which became bilaterally sensitive. Such a newly acquired behaviour of neurons is developing due to formation of a common system conducting the afferent impulsation from both sides of the body along somatic (sciatic) and visceral (pelvic) nerves to the only non-operated cortical area. It seems that the processes of compensation in both somatic and visceral systems conform to a general law.

481.2

ERRONEOUS REINNERVATION OF RAT VIBRISSAL FOLLICLE-SINUS COMPLEXES (F-SCs) AFTER SELECTIVE TRANSECTION AND REGENERATION OF DEEP VIBRISSAL NERVES. F.L. Rice, R.N. Strominger*, T.M. Mosconi* and I.R. Boneice*. Dept. of Anatomy, Albany Medical College, Albany, NY 12054.

Each F-SC in the rat mystacial pad is innervated from the infraorbital nerve by a single large deep vibrissal nerve (DVN) and several smaller superficial vibrissal nerves (SVNs). Each type of nerve provides unique proportions and orientations of sensory endings to different and segregated targets within each F-SC. Transection of the infraorbital nerve proximal to the mystacial pad results in apparently chaotic F-SC reinnervation (Renehan, W.E., J.Comp.Neurol., 249:429, 1986.) Either DVN and SVN afferents are misrouted at the site of the transection and are attempting to find their proper target, or they lost their target specificity. To test these possibilities, 23 DVNs were transected and anastomosed at their entry into the F-SCs. The SVNs were left intact. After a 1 to 3 month survival, the rats were anesthetized and fixed by perfusion. Normal and reinnervated F-SCs were prepared with the Winkelmann stain. Surprisingly, most if not all of the regenerated DVN afferents bypassed their normal targets and invaded the inner conical body which is normally a site of SVN innervation. Thus, regenerating DVN afferents innervate an inappropriate target that was not denervated. (Supported by NIH NIDR RO3 DE08734-01).

481.3

CHRONIC EFFECTS OF ADULT INFRAORBITAL (IO) NERVE CUT ON MONOAMINE CONTENT IN THE RAT SPINAL TRIGEMINAL NUCLEUS. B.G. Klein & W.D. Blaker. Dept. of Biomed. Sci., VA-MD Reg. Coll. Vet. Med., Virginia Tech, Blacksburg, VA 24061.

Cutting the IO nerve in adults produces long-term somatosensory reorganization in rat trigeminal brainstem nuclear complex (TBNC). Lesion-induced changes in monoamine systems may be a substrate for this reorganization, since these systems normally influence responses of TBNC neurons. Thus, we have used HPLC-ED to measure serotonin (5-HT) & norepinephrine (NE) levels in the IO region of subnuclei caudalis (SpVc) & interpolaris (SpVi) 76-79 days after unilateral adult IO nerve cut. Following decapitation, brains were quickly frozen & 340 um transverse cryostat sections were cut. Samples of 1.0 & 2.25 mm^2 were respectively sliced from the lateral border of SpVc & SpVi sections, in the IO region. Samples were taken from nerve cut & intact sides of each section in 9 lesioned rats & unilaterally in 10 normals. Location of samples was histologically verified. In SpVc, no differences were observed in 5-HT or NE levels among the lesioned, intact or normal sides. However, in SpVi, 5-HT and NE levels were increased by 46% & 60%, respectively, compared with the matched intact side. The data for SpVc are consistent with our previous finding that adult IO nerve cut does not alter the density of 5-HT immunoreactive varicosities in the superficial laminae. Analysis of monoaminergic fiber distribution within SpVi is in progress. Support: #88-187-CVM.

481.4

PLASTICITY IN THE RAT TRIGEMINAL SOMATOSENSORY PATHWAY: RETURN OF CORTICALLY-DEPENDENT BEHAVIOR FOLLOWING LESION OF MAIN V NUCLEUS. M.H. Friedberg*, S.M. Lee, M.G. Weisskopf*, and F.F. Ebner (SPON: N. Knuckey). Center for Neural Science, Brown University, Providence, RI 02912

The functional sensory contribution of the trigeminal subnucleus interpolaris (SpVi) to a cortically-dependent behavioral task was studied in seven Long-Evans rats following kainic acid lesions of the main trigeminal nucleus (MainV). The animals' ability to make a "jump-no jump" decision on the basis of vibrissa-transduced information was measured before, within 24 hr and after 24 hr following the MainV lesions.

Lesioned animals were unable to use vibrissa-transduced information to perform the gap-jump task during the initial 24 hr period. Thalamic recordings during this same period failed to reveal any neurons sensitive to vibrissae movements. Retesting each of the seven experimental animals after 24 hr demonstrated a marked increase in the success rate comparable to those of prelesion cases at gap distances shown to be dependent on integrity of the vibrissa somatosensory pathway. Subsequent destruction of SpVi after 10-30 d testing periods resulted in a permanent inability to use vibrissa-transduced information similar to the initial 24 hr period. In four control animals comparable injections of saline produced no behavioral or physiological deficits.

Our results agree with those of Rhoades et al. that lesions of MainV produce expanded receptive fields of ventroposteromedial (VPM) neurons. Our results show that an even more dramatic expansion occurs in the SI cortical region ("barrel field"). Despite this apparent loss of acuity the animal can use the sensory information mediated through the SpVi to make cortically-dependent decisions.

(Supported by NIH grant #NS-13031 and the Mathers Foundation)

481.5

CHANGES IN RECEPTIVE FIELD PROPERTIES OF RAT BARRELFIELD NEURONS FOLLOWING THALAMIC LESIONS. F.F. Ebner, M.A. Armstrong-James* and M.E. Diamond, Center for Neural Science, Brown Univ., Prov., R.I. 02912

In rats, two pathways conduct information from the mystacial vibrissae to the barrelfield (BF) cortex; one projects through the thalamic ventroposterior medial nucleus (VPm) to the center of cortical barrels, the other relays in the posterior group (PO) before terminating in the septa surrounding cortical barrels. Center (CRF) and surround (SRF) receptive fields of barrel field neurons were analyzed 2-30 days following excitotoxic NMDA lesions of VPm and/or PO. Following VPm lesions, 29% of BF neurons (N=38) responded to more than 8 vibrissae compared with 6% for controls, and 11 of 38 neurons had discontinuities in SRFs. Neurons also showed unusual latencies (very short or very long), abnormal spontaneous activity (too low in layer IV and too high in other layers), long lasting SRF responses (70-250 ms) with secondary discharges at longer latency (150-300 ms). Isolated "hot spots" in the SRF often equalled the response magnitude of the CRF, and responses typically consisted of long trains of impulses (bursts). In addition, repetitive stimulation (1/sec) often generated dramatically decremented or augmented responses.

Our interpretation is that lesions of the normally dominant VPm input to BF cortex lead to enhancement of the normally undetectable PO influences, permitting cortex to respond to sensory stimuli in a novel way. (Supported by NIH grant #NS-25907)

481.6

Plasticity in the barrel cortex of the adult mouse: temporarily increased GAD-immunoreactivity after sensory stimulation.

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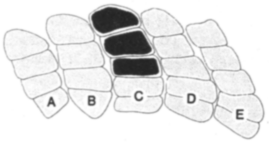
+Unitat de Biologia Celular, Facultat de Biologia, Universitat de Barcelona, 08071 Barcelona, Spain.



Activity-dependent regulation of neurotransmitters has been proposed as a mechanism for central plasticity. Sofar, observations have demonstrated such effects after sensory deprivation. We here report on the effects of increased sensory stimulation on GAD-immunoreactivity in the barrel cortex of the mouse. Four days of sensory stimulation of given sets of vibrissae on one side of the animal's muzzle, using the Lausanne whisker-stimulator (Melzer et al. 1985), resulted in an increase in GAD-immunoreactivity in the corresponding barrels (see Figure). These modifications consisted of an increase in the intensity of staining of puncta and cell bodies, as well as of an increase in the estimated numerical density of the puncta. In three out of four cases, the numerical density of cell bodies increased as well, even if slightly. After stimulation the effect wore off gradually until, five days after its arrest, GAD-immunoreactivity was restored to normal. These observations strongly suggest that GAD-synthesis increases as a function of peripheral sensory activity. Our companion report (Rao et al., this volume) shows the probable physiological consequence of such an increase in the behaving mouse, using the deoxyglucose method. Support: Swiss NSF 3100.009468.

481.7

Plasticity in the barrel cortex of the adult mouse: effects of prolonged whisker stimulation on acute stimulus evoked DG-uptake.

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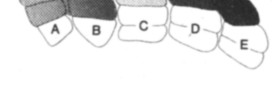

We investigated experience-dependent regulation of cortical activity in the whisker-to-barrel pathway of the adult mouse using the ^{14}C-deoxyglucose method. Under Nembutal anaesthesia, metal pieces were glued on left vibrissae C1-3; all other whiskers remained intact. The mice were exposed to magnetic field bursts while freely moving in the Lausanne whisker-stimulator (Melzer et al. 1985). Duration of stimulation: 1 day (n=4), 2 days (n=10) and 4 days (n=8), after which the metals were unglued from the stimulated whiskers. The arcs of vibrissae, of which these whiskers formed a part, were kept intact, whereas on the stimulated side all other whiskers were clipped. With this whisker configuration mice were allowed to explore, for 45 min, an object-filled cage, after having received a DG-injection. Non-stimulated animals, roaming the same cage with identical whisker configurations, served as controls (n=9). Autoradiograms of tangential sections through the barrelfield revealed a decrease of stimulus-evoked DG-uptake in the "pre-stimulated" barrels as compared to the other barrels of the same arc (see Figure). The effect was present only, and in *all*, pre-stimulated mice. This effect of prolonged periods of stimulation upon DG-uptake in a behaving animal shows the capacity of cerebral circuitry to adapt its local activity to sensory experience, and our companion report (Welker et al., this volume) suggests that GABAergic mechanisms may be at the root of this phenomenon.

Support: Swiss NSF 3100.009468.

481.8

CLASSICAL CONDITIONING OF A SPARED RAT VIBRISSA RESULTS IN BEHAVIORALLY SIGNIFICANT INCREASES IN FUNCTIONAL CORTICAL AREA. C.L. Hand, R.L. Craik, W.J. Carr & P.J. Hand. Emory U., Atlanta, GA 30322, Beaver College, Glenside, PA 19038, and University of Pennsylvania, Philadelphia, PA 19104.

To explore treatment interventions that maximize functionally-appropriate brain alterations, this study combined a bilateral spared vibrissa preparation with unilateral associatively-paired (AP) training in 8 rats. Subtotal deafferentation involved bilateral sparing of C3 vibrissae (SC3) before postnatal day 3. AP training (classically pairing vibrissa stroking with sugar water) of left or right SC3 was continued for 5 min/day for 60 days. Using the quantitative 2DG metabolic technique, results reveal a significant ($p<0.05$) increase in SC3/AP cortical area of 34.9% ($\pm$14.1,SE). Does increased cortical area mean enhanced sensory processing? Behavioral testing involving 5 days of 4 minute trials using a darkened, raised circular maze indicates that 6 of 8 (75%) rats traveled in the predicted direction preferring to use the SC3/AP-trained vibrissa in dark exploration. The data suggest that intervention resulting in significant functional cortical alteration also resulted in behavioral observations that can be quantified. Correlation of sensory training, changes in functional brain activity, and behavioral outcomes is therefore possible in the same experimental animal. NIH 22283-03.

481.9

ALTERATIONS IN CORRELATED ACTIVITY PARALLEL ICMS-INDUCED REPRESENTATIONAL PLASTICITY. H.R. Dinse, M.M. Merzenich Coleman Lab, UCSF, San Francisco CA, 94143

To investigate mechanisms underlying the remodeling of topographic representations by use, we have studied by means of simultaneous recordings correlation of unit response (CORR) at separated locations in the forepaw zone of SI in rats and in the hand representations of area 3b in owl and squirrel monkeys while inducing local cortical representational changes by intracortical micro stimulation (ICMS; cf. Recanzone & Merzenich, Neurosci Abst 14: 223, 1988). Control experiments revealed a distance dependence of CORR that dropped to a chance level with a separation of recording distances > about 300 microns. After ICMS, CORR doubled for recording sites separated by less than about 300 microns around the stimulation site, and rose well above control levels for loci separated out to more than 600 microns away from it. Correlograms were centered around zero tau, half-widths of the peaks were 10 to 20 ms.

This dramatic increase in CORR was restricted to cortical sectors over which representational changes were induced by ICMS. Receptive field (RFs) within these regions moved to overlap with the RF at the stimulation site by either shifting entirely, or increasing their size. Changes were assessed quantitatively by deriving PSTHs at sample skin locations using a skin stimulator. We found parallel time course for changes of CORR, RF-overlaps, equivalence of PSTH response magnitudes, and onset latencies. Changes were first seen after 15 to 30 min of ICMS. Steady state conditions were reached after 2 to 3 hours, and remained robust over several subsequent hours of study.

These studies suggest that temporal discharge coincidence plays an important role in the formation of functionally coupled cortical neuron groups and implicate these functionally groups in representational plasticity. They provide further evidence for a long term potentiation induced by ICMS.

Supported by NIH grant NS-10414, the Coleman Fund, HRI and the Max Kade Foundation

481.10

FREQUENCY DISCRIMINATION TRAINING ALTERS TOPOGRAPHICAL REPRESENTATIONS AND DISTRIBUTED TEMPORAL RESPONSE PROPERTIES OF NEURONS IN SI CORTEX OF ADULT OWL MONKEYS. G.H.Recanzone*, W.M.Jenkins, G.T.Hradek*, C.E.Schreiner, K.A.Grajski and M.M. Merzenich Coleman Laboratory, UCSF, San Francisco, CA 94143.

This study was initiated to define the changes in representation of a restricted skin surface in SI cortical fields arising from training in a flutter frequency discrimination task. Adult owl monkeys were trained to discriminate differences in tactile stimulus frequency using a go, no-go behavioral procedure. Stimulation was restricted to a small part of a single digit segment. The standard stimulus was 20 Hz. Discriminable flutter frequency differences declined with practice, with monkeys ultimately detecting 1-3 Hz differences.

The cortical representation of the stimulated and adjacent digits as well as that of the contralateral untrained hand was determined by defining receptive fields in several hundred microelectrode penetration sites in both hemispheres. Peristimulus time histograms at flutter frequencies used in the behavior were also derived at most sites. Five main results were obtained: 1) The cortical zone representing the stimulated skin expanded. 2) The average size of receptive fields extending over the practiced skin enlarged. 3) There was an expansion of cutaneous responses into a broad zone rostral to the physiologically-defined area 3b (presumably 3a) that usually represents 'deep' inputs. Multiple-digit and Pacinian receptive fields were predominant in this area. 4) Temporal response properties were differentially expressed in 3b and presumptive 3a for the skin region stimulated during the behavioral task. 5) These changes were not recorded in control animals in which the same stimuli delivered on the same schedule were behaviorally irrelevant. Research supported by NIH grants NS-10414,GM-07449, HRI and the Coleman Fund.

481.11

SENSORIMOTOR CORTEX LESIONS: TIME-DEPENDENT ANATOMICAL CHANGES SPECIFIC TO THE HOMOTPIC CORTEX. T. A. Jones* and T. Schallert; Psychology Dept. and Institute for Neurosciences, Univ. of Texas, Austin, 78712.

Previously, we have reported that there is a window of time following unilateral electrolytic lesions to the caudal forelimb region (CF) of the sensorimotor cortex during which the contralateral homotopic cortex (hCF) is especially vulnerable to damage (Jones, Barth, & Schallert, 1988). That is, a second lesion placed in the hCF within a certain time-period results in more extensive damage to the cortex relative to the first lesion. The present experiment examined the possibility that this vulnerability corresponds to time-dependent lesion-induced anatomical changes in the hCF. Following unilateral CF lesions, the thickness of the cortex was measured at 3, 7, 14 and 60 days postoperative at several positions within and neighboring the hCF. A transient increase in thickness was found relative to shams localized in those areas of the hCF containing large pyramidal cells in Layer V. This increased thickness was found when atypical and transient postural asymmetries in forelimb usage were observed and corresponds to a time at which the hCF is maximally vulnerable to damage (7 days). At 14 and 60 days, a reduction in thickness (but not a complete return to control levels) was found. This localized increase and subsequent decrease in cortical thickness may represent the initiation and stabilization of lesion- and/or behaviorally-induced structural changes in the hCF.Supported by NIH grant NS23964 awarded to T.Schallert.

481.12

IMMEDIATE CONSEQUENCES OF ULNAR NERVE TRANSECTION ON SOMATOSENSORY REPRESENTATION IN POST-SIGMOID GYRUS OF CAT: LIMITATIONS ON THE UNMASKING PROCESS. R.S. Waters, A. Oladehin*, E.F. Johnson*, C.X. Li*. Dept. of Anatomy and Neurobiology, Univ. of Tennessee, Memphis, Col. of Medicine., Memphis, TN 38163.

We report the consequences of sectioning the ulnar nerve on the functional organization of somatosensory cortex in cat. This study was undertaken to lay the groundwork for ongoing studies on the mechanisms underlying the unmasking process after peripheral denervation.

Sixteen adult cats were anesthetized with sodium pentobarbital, and a craniotomy and dura resection were performed to expose the post-sigmoid gyrus. The cortical surface was covered with warmed silicon fluid, and multi-unit responses were recorded from a carbon-fiber electrode inserted into the primary somatosensory cortex. The receptive field was recorded, and the location of the recording site was marked on a photograph of the cortical surface. The relationship between the electrode location and the corresponding receptive field was used to generate a cortical map. Following forepaw mapping, the ulnar nerve was exposed below the elbow and sectioned. The post-sigmoid gyrus was remapped immediately after nerve section, and again at 12 h and 36 h in the same animal.

Immediately after nerve cut we observed a large unresponsive region in the cortical location formerly occupied by the 5th digit and the ulnar side of the 4th digit. At 12 h after nerve cut, new responses were recorded from electrode penetrations in the outermost portion of the unresponsive cortex. Expansion into the unresponsive region from digits 3 and 4 and the forearm was clearly evident. At 36 h further expansion into unresponsive cortex was observed, but many unresponsive sites still remained.

These results further document the unmasking phenomenon as reported in other species, but place limitations on the area of cortex subject to immediate reorganization. (Supported by NSF Grant BNS 88-02766.)

481.13

SEQUENCE OF CHANGES IN THE PRIMARY SOMATOSENSORY CORTICAL REPRESENTATION OF THE BODY FOLLOWING COMPLETE DEAFFERENTATION OF THE FORELIMB IN ADULT CATS. R.J. Schneider[1], S.S. Leclerc[2], R.S. Waters[3], I. Salimi*[4], R.W. Dykes[4]. [1]Lab of Neuropsychology, NIMH, Bethesda, MD, [2]Dept. of Neurology and Neurosurgery, McGill Univ., Montreal, Que., [3]Dept. of Anatomy and Neurobiology, Univ. of Tennessee, Memphis, Col. of Med., Memphis, TN, [4]Dept. of Physiology, Univ. of Montreal, Montreal, Que.

In this report we document the changes that occur in the functional organization of the cat primary somatosensory cortex at selected times following denervation of the nerves serving the forelimb. This information will provide the groundwork for subsequent studies of the role neurotransmitters play in neocortical plasticity.

Thirty-two cats were used. Ten maps of the primary somatosensory cortex were obtained in unoperated animals. In the remaining animals, the median, radial, and ulnar nerves were sectioned under general anesthesia. At 2 days, 1, 2, 4, 6, 8 weeks, or 1 year later, the animals were reanesthetized with sodium pentobarbital, and the organization of the body representation in the post-sigmoid gyrus was studied with low impedance carbon-fiber electrodes. The location of the electrode penetration was marked on a photograph of the cortical surface, and the receptive field activating neurons at that site was described in terms of modality, submodality, and threshold.

Two days following deafferentation, we observed a large unresponsive region in the former forelimb cortical representation. Within this region, a limited number of sites could be driven by stimulation of the upper arm, shoulder, and/or neck. By the end of the first week, a larger number of cortical sites could be driven by tactile stimulation, and by the end of the second week, the extent of responsive and unresponsive regions were nearly of equal size. At one month, the extent of unresponsive cortex was reduced further, but at one year, a significant amount of unresponsive cortex still remained.

(Supported by Grants from NSF, MRC, and FCAR).

481.14

THE RESULT OF PARTIAL DENERVATION OF FORELIMB ON THE SOMATOSENSORY REPRESENTATION IN THE POST-SIGMOID GYRUS IN THE CAT. S.S. Leclerc*[1], R.J. Schneider[2], R.S. Waters[3], P. McKinley[4], C. Chau*[4], R.W. Dykes[5]. (Spon: Bertorini, T.E.) [1]Dept. of Neurology and Neurosurgery, McGill Univ., Montreal, Que. [2]Lab of Neuropsychology, NIMH, Bethesda, MD, [3]Dept. of Anatomy and Neurobiology, Univ. of Tennessee, Memphis, Col. of Med., Memphis, TN, [4]School of Physical and Occupational Therapy, McGill Univ., Montreal, Que.,[5]Dept. of Physiology, Univ. of Montreal, Montreal, Que.

In addition to those animals described in the preceeding abstract only one or two of the forelimb nerves were cut in 5 additional cats. One week, one month, or one year later, the animals were reanesthetized with sodium pentobarbital and the somatosensory cortex was exposed through a craniotomy over the frontal pole. The cortex was covered with silicon fluid, and single and multi-unit responses were recorded in primary somatosensory cortex using carbon-fiber electrodes. The body was searched for receptive fields that would activate the cortical site being studied.

In comparison to those animals having a complete denervation of the forepaw, the amount of unresponsive cortex found in the partially denervated animals in the region originally serving the forepaw was smaller, but the area was never completely reactivated by inputs from other body parts. There were fewer unresponsive sites when both the radial and median nerves were left intact than when only the median nerve alone was left intact, however, again there remained cortex that could not be driven from the periphery. Unusually large receptive fields were frequently found in deafferented cortex about one month after the forelimb denervation. After one year these large receptive fields were uncommon; receptive fields in this region formerly serving the forelimb could be activated from a well-defined body site. At one year the cortical areas unresponsive to somatic stimuli were more irregularly distributed throughout the representation than was the case at earlier times following deafferentation. (Supported by Grants from MRC, FCAR, and NSF).

481.15

ACTIVITY-DEPENDENT PLASTICITY IN THE MATURE RAT GENICULO-STRIATE SYSTEM DURING MONOCULAR RETINAL BLOCKADE. G.A. Thurlow and R.M. Cooper. Behavioral Neuroscience Research Group, Psychology Dept., University of Calgary, Calgary, Alberta, Canada, T2N 1N4.

We examined the effects of monocular loss of retinal activity on 2-deoxyglucose (2-DG) uptake in the mature hooded rat geniculostriate visual system. Rats were subjected to either short-term (24 h) or long-term (21 - 90 d) monocular TTX injections and exposed to a visually stimulating environment during 2-DG uptake. As compared to short-term TTX-rats, 2-DG uptake increased in binocular area 17 of the long-term TTX-rats. In a third group, TTX was monocularly injected for 30 or 60 d and, 24 h before 2-DG, retinal activity was binocularly eliminated. Complete loss of input resulted in binocular area 17 depression greater than that seen in the short-term TTX group - indicating that the increases which occurred during long-term retinal blockade were dependent upon retinal activity from the non-TTX eye. LGNd 2-DG label demonstrated that the cortical shifts were reflections, at least in part, of changes occurring subcortically in the binocular geniculate. During long-term monocular retinal blockade in the mature rat, an activity-dependent shift in ocular influence occurs in the geniculostriate visual system, increasing the metabolic effect of input from the opposite eye.

481.16

RETINAL INPUT MODULATES THE PRESENCE OF SEROTONIN-IMMUNOREACTIVE CELLS IN THE HAMSTER'S SUPERIOR COLLICULUS. R.W. Rhoades, C.A. Bennett-Clarke, N.L. Chiaia and R.D. Mooney. Dept. of Anatomy, Medical College of Ohio, Toledo, OH 43699.

The stratum griseum superficiale (SGS) of the hamster's superior colliculus (SC) contains a substantial number of cells that are recognized by polyclonal antisera directed against serotonin (Inc Star and Accurate). These neurons can be seen in animals that are pretreated with pargyline only, but are much more numerous after pretreatment with both colchicine and pargyline. Pretreatment with reserpine causes the complete disappearance of all serotonin immunoreactive (SI) cells in the hamster's SC. In this experiment, we asked whether the presence of SI neurons in the hamster's SC could be modulated by the visual input to this nucleus. Adult hamsters were anesthetized with sodium pentobarbital and either one or both eyes were enucleated. One day to 2 months later, hamsters were pretreated with pargyline and colchicine, killed, and tissue was processed for SI. In hamsters that sustained removal of one eye, there was a virtually complete loss of SI cells in the ipsilateral SC (i.e. that which retained the bulk of its normal input from the contralateral retina). This effect was apparent within 3 days after enucleation and remained apparent for at least 2 months. Removal of both eyes from adult hamsters caused no significant depletion of SI neurons in either SC. Silencing of retinal activity in one eye for 3 days via injection of tetrodotoxin resulted in no significant change in the number of SI cells in either superior colliculus. These results indicate that the presence of SI in SC neurons can be altered by an "imbalance" in retinal input. This imbalance does not appear to involve retinal activity and results in a reduction in SI in cells that retain their input from the contralateral retina.

Supported by EY 04170, EY 08015, BNS 85 00142, and funds from the State of Ohio Research Challenge.

481.17

GAP-43 EXPRESSION IN REGENERATING ADULT OPTIC FIBERS IN VITRO. J. Miotke*, R.L. Meyer and L.I. Benowitz (SPON: G. LeBlanc). Developmental Biology Center, Univ. Calif., Irvine, CA 92727 and Dept. Psychiatry, Harvard Med. School, Belmont, MA 02178.

We previously reported that when the optic nerve is crushed in an adult mouse and 1wk later the retina explanted onto laminin, neurites originating from ganglion cells extend onto the substrate as early as 24h. If the optic nerve is not crushed, neurites begin to grow at about 4d. In this study, the expression and distribution of GAP-43 was examined using immunohistochemistry. In explants with prior nerve crush, all neurites were strongly positive as early as 24h and remained positive for up to 1m. Within the explants, numerous processes and some cell bodies were also positive. In explants without crush, no positive processes or cell bodies could be detected at 24 but they were seen at 4-6d. Strongly positive neurites were also seen at this and all later times. At 1-6d, GAP-43 was distributed throughout each neurite including the growth cone and fine filopodial side branches. By about 2wk, there was a notable accumulation of reactivity in dilations which were distributed periodically along the shaft and were especially numerous in the fine terminal-like branches that formed at the end of the neurite at these and later times. We conclude that adult neurons which normally have little GAP-43 can express GAP-43 following axotomy and can develop and maintain this response in vitro. The existence of GAP-43 in these neurites also indicates that they are axons. (Supported by NIH NS26750)

481.18

SYNAPSE FORMATION IN THE SUPRAOPTIC NUCLEUS (SON) IN VITRO: EFFECTS OF OSMOTIC STIMULATION. B.K. Modney and G. I. Hatton. Psych. and Neurosci. Michigan State Univ., E. Lansing, MI 48824

Magnocellular neurons in the rat SON alter their ultrastructural morphology in response to dehydration of the animal in vivo. To determine if similar changes occurred in vitro, horizontal hypothalamic slices that contained the SON were incubated in either normal (290 mOsm/kg, n=9) or high (340 mOsm/kg, n=8) osmolality medium. Electrical activity was monitored extracellularly from samples of neurons and mean firing rates were obtained. A significant increase in mean firing rate was found in the slices incubated in high osmolality medium. Ultrastructural evaluation revealed that incubation in such medium for 4-5 hours resulted in the formation of new multiple synapses (i.e., a single terminal contacting 2 or more somata or somata and dendrites). These results demonstrate the suitability of this slice preparation for further studies of the mechanisms through which the SON is reorganized. The rapidity of this response suggests that pre-existing terminals either within or around the SON are forming these new synapses. These synapses are thought to play an important role in the overall responsiveness of the SON to osmotic challenge. Supported by NIH NS 09140.

481.19

RAPID MORPHOLOGICAL CHANGES IN THE SUPRAOPTICO-NEUROHYPOPHYSIAL SYSTEM OF THE PERFUSED RAT BRAIN. C.D. Tweedle, K.G. Smithson and G.I. Hatton. Neurosci. Prog. , Mich. State Univ., E. Lansing, MI 48824.

The magnocellular neurons of the rat supraoptic nucleus (SON) alter their morphology in response to a variety of activating stimuli applied *in vivo*. To determine how rapidly these changes may occur we transcardially perfused anesthetized rats with oxygenated 37 °C tannic acid (0.5 -1.0 %) containing media of either elevated K+ (50 mM) for 10 minutes or elevated osmolality (340 mOsm) for 20 minutes. Ultrastructural analysis revealed increases in bundling of dendrites, multiple synapses onto dendrites, and terminal size in the neurohypophysis. These changes are similar to those observed *in vivo* under activating stimuli. No such changes were seen in rats perfused with tannic acid-containing media having normal K+ (5.6 mM) or osmolality (310 mOsm). Similar results have been obtained in pilot studies using such media without tannic acid. The rapidity of these responses (including synapse formation) with this procedure indicates that it may be a useful technique for future studies on glial- or neuroplasticity and also that the newly formed dendritic synapses must be of local origin. Supported by NS 09140 and a fellowship from the Medical Scientist Training Program to KGS.

482.1

COMPARISON OF STRIATAL EXTRACELLULAR DA LEVELS IN AWAKE AND ANAESTHETIZED RATS USING IN VIVO MICRODIALYSIS.

M.E. Hamilton, A. Mele*, and A. Pert. NIMH, BPB, Bethesda, MD. 20892.

A major concern for microdialysis users is that the anaesthetic used in the preparation may compromise neurotransmitter function. A 3 mm microdialysis probe was inserted unilaterally into the anterior striatum of male, Sprague-Dawley rats (300-350 g). Probes in the "awake" rats that had been previously implanted with 19 ga. guide cannulae were attached to a fluid swivel and rats were placed in a circular Plexiglas activity chamber. Chloral hydrate-anaesthetized rats were stereotaxically positioned. Basal DA levels were not significantly higher in anaesthetized rats. D-Amphetamine (AMPH: 0.25 mg/kg, s.c.) produced similar elevations of DA (approx. 230 %) in both groups. In anaesthetized rats, however, this increase persisted for at least 2 h, whereas recovery had occurred by this time in awake rats. A reduction in DOPAC levels by AMPH was more pronounced in anaesthetized rats and recovery to predrug levels was retarded. Similarly, HVA levels following AMPH were consistently lower in anaesthetized rats. These findings may reflect a generalized metabolic reduction by the anaesthetic. The similarity in initial responses to AMPH in both groups suggests that at least for examination of the effects of DA stimulants, the simpler anaesthetized preparation may not be cause for concern.

482.2

SENSITIZATION OF DOPAMINE RELEASE TO INJECTIONS OF COCAINE AS MONITORED BY IN VIVO MICRODIALYSIS.

R.W. Keller, Jr., I.M. Maisonneuve*, J.N. Carlson, S.D. Glick. Dept. of Pharmacology & Toxicology, Albany Medical College, Albany, NY 12208

We have examined changes in the levels of dopamine (DA) and its metabolites in the extracellular fluid of the striata of freely-moving, male, Long-Evans rats in response to I.P. injections of 20 mg/kg cocaine. Dialysis probes (Carnegie Med.) were lowered into previously-implanted guide cannulas and perfused at 1 ul per min. 20-min samples were assayed by HPLC-EC for DA, DOPAC, HVA and 5-HIAA. Baseline samples were collected beginning 2, 10, or 22 hrs after probe insertion. Saline or cocaine was injected 2 hrs later after a stable baseline was obtained. All rats were reexamined one week later; in each case baseline collection began 2 hrs after probe insertion and cocaine was injected 2 hrs later.

Basal extracellular fluid levels, estimated from pre-implantation calibration of the probes, were: 8.2nM DA, 6.9uM DOPAC, 5.3uM HVA and 2.8uM 5-HIAA. A 6-fold increase in DA was observed after cocaine while DOPAC, HVA and 5-HIAA showed modest decreases. We also studied infant rats (10-14 days) and found lower basal levels of all compounds and a more prolonged release of DA in response to cocaine. In adult rats, one week after cocaine, basal DA doubled, while it was unchanged in saline-injected controls. The increase in DA levels in response to the second administration of cocaine was also enhanced. Thus a single exposure to cocaine appears sufficient to produce a sensitization of DA release measurable in vivo. [Support DA-03817]

482.3

USE-DEPENDENT MODULATION OF THE NIGROSTRIATAL PATHWAY.

P. Miu* and J.W. Commissiong. (SPON: R. Chase). Dept. of Physiol., McGill Univ., 3655 Drummond St., Montreal, Canada H3G 1Y6.

Nigrostriatal dopaminergic cells were studied using microdialysis and single unit extracellular recording techniques. Female Sprague Dawley rats (220-250 g) were anesthetized with urethane (1.5 g/kg i.p.) and a dialysis probe inserted into the striatum, while a glass micropipette monitored activity in the zona compacta of the substantia nigra. Electrical stimulation of the medial forebrain bundle (MFB) at 1, 5, 10 or 15 Hz consistently reduced the firing rate of the dopaminergic cells (N = 13), but not of the non-dopaminergic cells. Concomitantly, the microdialysis data showed a reducted efflux of DOPAC and HVA during stimulation (Control: 66 pmol/20 min; Stimulated: 33 pmol/20 min). Infusion of TTX into the MFB, and subsequent blockade of nerve conduction caused an increase in the metabolism of DA in the striatum (DOPAC and HVA were increased by as much as 300% and 700% respectively). The data suggest that DA metabolism may be reduced when the firing rate of dopaminergic neurons is increased, and increased when nerve conduction in the MFB is suppressed. Therefore, there may not be a causal link between increased firing of dopaminergic neurons, increased release of DA, and increased metabolism of DA. Supported by the MRC of Canada.

482.4

CORRELATION OF PHENCYCLIDINE (PCP)-INDUCED STIMULATION OF LOCOMOTOR ACTIVITY WITH RELEASE OF ENDOGENOUS DOPAMINE IN VITRO IN TWO INBRED MOUSE STRAINS.

I.B. Finn*, L.P. Dwoskin, T.W. Seale, and J.M. Carney* (SPON: W.R. Martin). Dept. Pharmacol., Univ. KY Col. Med. and Pharmacy, Lexington, KY 40536, and Dept. Pediat., Univ. OK Hlth. Sci. Ctr., Oklahoma City, OK 73104.

Phencyclidine (PCP) (0.1-10 mg/kg, i.p.) produced a robust stimulation of locomotor activity (LA) in DBA/2J mice, with only slight stimulation in C57BL/6ByJ mice. Striatal slices from each strain were superfused for 30 min with Kreb's buffer containing PCP. The amounts of endogenous DA and its major metabolite, dihydroxyphenylacetic acid (DOPAC) in the superfusate were determined by HPLC-EC. Basal release of neurotransmitter prior to exposure to PCP was not different between strains. PCP (10-4M) resulted in a greater amount of DA (540 $\pm$ 60 vs 390 $\pm$ 40 pg/ml/mg) and a lesser amount of DOPAC (4060 $\pm$ 580 vs 6970 $\pm$ 460 pg/ml/mg) in superfusate from DBA/2J as compared to C57BL/6ByJ striatal slices, respectively. Therefore, the differential PCP-induced stimulation of DA release between the inbred strains of mice correlates with the differential stimulation of locomotor activity. Supported in part by grant DA04028 and NIDA contract 271-87-8133 (J.M.C.) and Univ. KY Med. Ctr. Research Fund (L.P.D.).

482.5

BRAIN BIOGENIC AMINES AND METABOLITES IN RATS SUBJECTED TO CROSS-COUPLED MOTION.

J.O. Owasoyo, M.M. Akmal and C.A. Walker. UAPB Research Center, University of Arkansas at Pine Bluff, Pine Bluff, AR 71601.

It is of wide interest to better understand physiologic factors that contribute to motion sickness in man. Therefore, the purpose of this study was to examine brain neurochemical changes that may accompany motion sickness by determining brain dopamine (DA), 3,4-dihydroxyphenylacetic acid (DOPAC), 5-hydroxytryptamine (5-HT) and 5-hydroxyindoleacetic acid (5-HIAA) in rats subjected to cross-coupled motion. Adult, male, Fischer 344 rats were used in this study. Control and sham animals, as well as animals (n=6) subjected for 20 min to cross-coupled motion were sacrificed at 30, 60 and 120 min after exposure to motion. The brain was removed and dissected into cortex, medulla and cerebellum for HPLC analysis of DA, DOPAC, 5-HT and 5-HIAA. Exposure to motion resulted in a significant increase in the DOPAC and 5-HIAA levels as well as an increase in the 5-HT concentration of the cortex and medulla. No change in the levels of biogenic amines or metabolite was observed in the cerebellum. These findings suggest that biogenic amine levels in the cortex and medulla may be involved in the effect of cross-coupled motion (Performed at NCTR and supported by NASA grant #NAG 2-427).

482.6

EFFECTS OF ACUTE AND CHRONIC HALOPERIDOL AND CLOZAPINE ON DOPAMINE RELEASE IN THE FRONTAL CORTEX AND STRIATUM.

M. Egan*, F. Karoum and R.J. Wyatt. Neuropsychiatry Branch, NIMH Neurosciences Center at St. Elizabeths, Washington, D.C. 20032.

The accumulation of 3-methoxytyramine (3MT), a reflection of dopamine release, was measured in the frontal cortex (Fx) and striatum (ST) following acute and chronic clozapine (CLOZ) and haloperidol (HAL) treatment. Rats received HAL (0.4 mg/kg), CLOZ (10 mg/kg) or vehicle (VEH) i.p. daily for 28 days, and were killed by microwave 10 min after pargyline (65 mg/kg) and 1 hr (CH-1hr) or 24 hr (CH-24hr) after final drug injection. 3MT was measured by mass fragmentography.

In the Fx, a single dose of CLOZ or HAL following chronic VEH elevated 3MT ($p<.05$). In the CH-1hr group, both drugs elevated 3MT, although less so than the single dose ($p<.05$), suggesting partial tolerance. In the CH-24hr group, 3MT returned to baseline. In the ST, after chronic VEH, HAL increased 3MT. There was no tolerance for the CH-1hr group; with the CH-24hr group, 3MT returned to baseline. CLOZ, did not significantly increase ST 3MT after a single or a CH-1hr dose; however, for CH-24hr dosing, 3MT was well below baseline (40%, $p<.05$). It appears that there is partial tolerance for both CLOZ and HAL in the Fx. In the ST, there is no tolerance to HAL. In contrast, CLOZ fails to increase ST 3MT above baseline, but decreases the 24 hr baseline. The clinical significance of these findings will be discussed.

482.7

INTRACAUDATE R(-)N^6-(2-PHENYLISOPROPYL) ADENOSINE (R-PIA) EFFECTS ON EXTRACELLULAR CAUDATE DOPAMINE (DA) AS MEASURED BY MICRODIALYSIS. M.E. Morgan, B.K. Yamamoto and R.E. Vestal*. VA Med. Ctr., Boise, ID 83702, Northeastern Ohio Univ., Rootstown, OH 44272, Univ. Wash. School Med., Seattle, WA 98195

Adenosine (ADO) has been shown to modulate basal ganglia function. Microdialysis was used to simultaneously infuse R-PIA, an ADO A_1 agonist, into caudate and to measure extracellular levels of several compounds in freely moving rats. Dialysis probes were continuously perfused with Dulbecco's phosphate buffer containing 1.2 mM $CaCl_2$, pH 6.0, at a rate of 2.5 μl/min. Dialysate samples were collected every 20 min, split into two aliquots and analyzed by HPLC EC for DA, 3,4-dihydroxyphenylacetic acid (DOPAC), homovanillic acid (HVA) and 5-hydroxyindoleacetic acid (5-HIAA) or aspartate, glutamate, taurine and GABA. After extracellular caudate DA levels stabilized, R-PIA was infused for 20 min followed by a 120 min drug washout. R-PIA, 5.0 nmoles, significantly increased DA levels by 229±26% baseline (mean±SE, n=6), but did not alter DOPAC, HVA or 5-HIAA levels, 96±1, 90±4, 88±4% baseline, respectively. At 40 min after drug washout, DA levels had decreased to 60±5% of baseline but had not return to baseline values by 120 min. DOPAC, HVA and 5-HIAA gradually decreased during the washout period. Amino acid levels were not altered. These data are the first to show that R-PIA induces an increase in extracellular caudate DA in freely moving rats.

482.8

EFFECTS OF THE BENZODIAZEPINE MIDAZOLAM ON EXTRACELLULAR DOPAMINE CONCENTRATIONS IN THE NUCLEUS ACCUMBENS AND STRIATUM. J.M. Finlay, G. Damsma, D. Wenkstern*, H.C. Fibiger. Div. of Neurol. Sci., Dept. of Psychiatry, Univ. of British Columbia, Vancouver, BC, V6T 1W5.

It has been suggested that benzodiazepines indirectly excite dopamine (DA) neurons within the ventral tegmental area and substantia nigra of the rat (cf. O'Brien & White, 1987). Given this, *in vivo* microdialysis was used to examine the effects of midazolam (1 and 5 mg/kg; sc) on extracellular DA concentrations in the striatum and nucleus accumbens of freely moving rats. Midazolam decreased DA in the nucleus accumbens (to 65% of baseline) in a time dependent manner such that the maximal effect was observed approximately 1.5 h postinjection followed by a return to baseline values within 4-5 h postinjection. Striatal DA concentrations were unaffected by the drug treatment. In contrast to the observation that benzodiazepines can increase the electrophysiological activity of DA neurons, the present results indicate that benzodiazepines either decrease or have no effect on extracellular DA within the terminal region of these neurons. This discrepancy suggests that 1) benzodiazepines have different effects on the activity of DA neurons in chloral hydrate anaesthetized (electrophysiological experiments) and freely moving rats (present experiment) or 2) an increase in activity of DA neurons at the level of the cell bodies may not necessarily be associated with an increase in extracellular DA at the level of the terminals.

482.9

INCREASED DA RELEASE IN PREFRONTAL CORTEX OF AWAKE FREELY MOVING RATS BY THE ANXIOGENIC BETA-CARBOLINE FG-7142 AS MESURED BY MICRODIALYSIS. C. W. Bradberry and R.H. Roth.

Previous work from this laboratory has demonstrated that the anxiogenic beta-carboline FG-7142 selectively increases indices of dopamine metabolism in the medial prefrontal cortex (mPFC) of the rat (DOPAC, HVA, in vivo tyrosine hydroxylation). In order to directly examine the effects of FG-7142 on dopamine release in mPFC, we have employed microdialysis in the awake rat. Because the dopaminergic innervation of this region has been shown to be extremely sensitive to environmental stressors, the animals were implanted with 23 gauge guide cannulae above the mPFC, and then habituated to the handling necessary for implanting the dialysis probe, and to the experimental environment. The probe itself was extremely lightweight and coupled to the guide cannula by a small section of polyethylene tubing. Only the fused silica inlet and outlet lines ran to the liquid swivel above the animal, allowing complete freedom of movement. At least 5 days following cannulae implantation, dialysis probes were inserted early in the morning and perfused for six hours to establish a stable baseline. At this point, the recovery of DA was .124±.015 fmols/microliter collected at a flowrate of 2 microliters/min. Administration of FG-7142 (20 mg/kg i.p.) caused a short duration increase in DA which peaked at 60% of preinjection value in the second 20 min fraction following injection. The time course of this effect agrees with earlier results from this laboratory in which whole tissue biochemistry was studied. Supported by MH 14092.

482.10

INVESTIGATION OF ELECTRICALLY STIMULATED RELEASE FROM THE LOCUS COERULEUS-NOREPINEPHRINE SYSTEM IN THALAMUS USING IN VIVO VOLTAMMETRY. M.H.B. Ghasemzadeh*, P. Capella*, R.N. Adams. Dept. of Chemistry, University of Kansas, Lawrence KS 66045.

Nafion-coated carbon fiber electrodes (CFE) were used to study the locus coeruleus (LC)-norepinephrine (NE) system. We have electrically stimulated (10 sec., 50-100 µA) both LC and its ascending dorsal bundle, and have recorded a "fast" signal (30-40 sec. duration) followed by a "slow" signal (2-5 min. duration) in the anterior ventral nucleus of thalamus in anesthetized rats. We believe the "fast" signal to be composed of primary catecholamine(s), and the "slow" signal, catecholamine metabolite(s): (1) the location of the stimulating electrode and CFE must be in known NE tracts and terminal fields to observe the release; (2) the applied potential was sufficient to oxidize catecholamines, not indoleamines; (3) inhibition of tyrosine hydroxylase reduced both signals by 50-70%; (4) inhibition of monoamine oxidase eliminated the "slow" signal and increased the "fast" signal 3-4 fold; (5) inhibition of NE uptake by desipramine increased and broadened the fast signal by 2-3 fold. This is the first report of real time, in vivo monitoring of release and metabolism of neurotransmitters from the LC-NE system.

482.11

A RAPID MICROSAMPLING TECHNIQUE DESIGNED FOR THE INTERFACE BRAIN SLICE PREPARATION. R. Shiehkattar*, L. Bibbs* and R.N. Adams (SPON: Nancy A. Dahl). Dept. of Chemistry and Biochemistry, University of Kansas, Lawrence, KS 66045.

A technique has been described for microsampling of the thin liquid film of perfusion fluid from the top of the conventional interface brain slice preparation. 10 to 15 uL samples were withdrawn by glass capillaries pulled to a tip diameter of 50-100 um and gently blown into the eppendorf tubings. The samples were immediately analyzed with HPLC-EC for the biogeric amines, their metabolites, ascorbic and uric acid.

Our results indicate that the sampling provides a reproducible readout of endogenous amines and their metabolites throughout the slice lifetime. In addition, the utility of the technique for the rapid sampling is demonstrated by monitoring the K^+ stimulated release of dopamine from the coronal slices of the rat striatum. Samples were collected as fast as 15 sec to reveal the entire overflow profile for dopamine. Basal and the stimulated release of the amino acids have also been measured in the same slices.

This new rapid sampling technique could be utilized to measure any number of endegenous compounds for which the analytical readout is available.

482.12

FACTORS CONTRIBUTING TO THE *IN VITRO* DETECTION OF DOPAMINE USING STEARATE-MODIFIED GRAPHITE PASTE ELECTRODES. **C.D. Blaha[1,2], M.E. Jung[1], A.G. Phillips[1], and H.C. Fibiger[2]. Depts. of Psychology[1] and Psychiatry[2], University of British Columbia, Vancouver, BC, Canada, V6T 1Y7.**

Stearate-modified electrodes (SGE) exhibit a selective electrochemical response to DA as evidenced by chronoamperometric techniques (Brain Res. Bull. 10:861, 1983). Conventional graphite paste electrodes (CGE, mineral oil and graphite; 3:2 wt.:wt.) cannot resolve DA metabolites (DOPAC) or ascorbic acid (AA) from DA. Linear sweep voltammetry (LSV) and 1 sec pulse chronoamperometry (CA) were used to examine the properties of electrodes with different concentrations of stearate (0, 0.1, 0.2 and 0.4 g/vol) and the effects of brain treatment and changes in medium temperature (22-37°C).

The data indicate that under simulated *in vivo* conditions (ie. 37°C and exposure to brain tissue) (1) the greatest sensitivity to DA (50 nM; CA) was obtained with 0.1 g/vol stearate, (2) sensitivity to DA was 4x greater at SGEs than CGEs, (3) sensitivity to DA was enhanced 2x (pre vs post-brain treatment) at the SGE but markedly attenuated at the CGE, (4) electrocatalysis of DA by AA decreased from 5x at a scan rate of 10 mV/sec to 1.5x at 500 mV/sec, saturated at concentrations of 100 to 1 AA/DA and (5) was absent using CA. Results confirm that SGEs can resolve DA from DOPAC or AA and provide further support for their use to detect DA efflux *in vivo*.

482.13

PRESYNAPTIC INHIBITION OF STRIATAL DOPAMINE RELEASE IS PREVENTED BY p-BROMOPHENACYL BROMIDE. W.A. Cass*, T.V. Dunwiddie, F.A. Fitzpatrick* and N.R. Zahniser (SPON: M.D. Womble). Dept. of Pharmacology, Univ. Colorado Hlth. Sci. Ctr., Denver, CO 80262.

The mechanisms underlying presynaptic receptor modulation of dopamine (DA) release have not been clearly defined. Modulation of endogenous DA release (assayed by HPLC-EC) from rat striatal slices was measured following electrical stimulation (60 pulses, 1 Hz). Slices were superfused with Krebs' buffer containing 10 uM nomifensine at a rate of 1 ml/min. One ml samples were collected before and after the two periods of stimulation. Drugs were added 30 minutes before the second period of stimulation. Adenosine (50 uM) inhibited evoked DA release by 30-40%. This effect was apparently mediated via an A1 adenosine receptor because the A1 specific agonist N^6-cyclohexyladenosine (100 nM) produced a similar inhibition while the A2 specific agonist CGS 21680 (100 nM) had no effect. The inhibition of DA release caused by adenosine was abolished by co-perfusion with the adenosine receptor antagonist 8-phenyltheophylline (10 uM). p-Bromophenacyl bromide (BPAB) irreversibly inhibits phospholipase A2 and thus prevents release of arachidonic acid (AA) from membranes. Co-perfusion with BPAB attenuated, in a dose dependent manner, the inhibition of evoked DA release produced by adenosine. 10 uM BPAB produced a maximal effect. BPAB (10 uM) by itself tripled the basal release of DA, suggesting a role for AA metabolites in the control of basal release of DA, as well as in the modulation of stimulated release. The AA cascade may be a general mechanism involved in the presynaptic inhibition of striatal DA release, as BPAB also abolished the inhibition of DA release caused by the D-2 DA receptor agonist N-0437 (3 nM). Supported by USPHS NS26851 and AA07464.

482.14

USE OF INTRASTRIATAL MICRODIALYSIS IN THE STUDY OF THE MECHANISM(S) UNDERLYING DOPAMINE RELEASE BY BOMBESIN, NEUROPEPTIDE Y AND d-AMPHETAMINE. Z. Meral. Psychology & Pharmacology, University of Ottawa, Ontario, Canada. K1N 9A9.

Bombesin (BN) and neuropeptide Y (NPY) pharmacologically affect motor activity in rats. The caudate-putamen (CPu) and the nucleus accumbens (Acb) represent behaviorally relevant areas, rich in dopaminergic (DA) input, BN binding sites and NPY levels. The effect of BN and NPY on DA and its metabolites (DOPAC, 3-MT and HVA) were compared to those of d-amphetamine (d-AMPH). Sragues-Dawley rats were implanted with microdialysis assemblies with guide shafts aimed at the Acb and the CPu (Brain Microdevices Inc., Box 410, Station A, Ottawa, K1N 8V4). After steady baseline, BN (40 ug), NPY (30 ug) or d-AMPH (40 ug) was pulsed through the probe (30 ul/20 min) and samples collected for 2 hr. At both sites, BN and NPY increased levels of DA. This was followed by smaller but more prolonged increases of DOPAC and HVA. d-AMPH markedly increased DA but decreased DOPAC and HVA levels. 3-MT levels that were barely detectable at baseline, increased sharply after d-AMPH but not after BN or NPY. Thus both BN and NPY can increase the extracellular levels of DA most likely by facilitating its release and not by blocking its reuptake. (Supperted by MRC).

482.15

PROLACTIN MODULATES DOPAMINE RELEASE FROM THE CORPUS STRIATUM THROUGH A SPIPERONE BINDING SITE IN THE ABSENCE OF EXTRACELLULAR CALCIUM. N.J.Laping, D.E.Dluzen, and V.D.Ramirez. Department of Physiology and Biophysics, University of Illinois, Urbana, IL 61801.

In vitro basal and amphetamine (AMPH) stimulated dopamine (DA) release from corpus striatum (CS) fragments of 3-4 month old male rats was measured in the presence of 10^{-7}, 10^{-6}, and 10^{-5}M ovine prolactin (PRL). Additional conditions included 10^{-5}M PRL in calcium-free medium, 10^{-6} spiperone, and 10^{-5}M PRL in the presence of 10^{-6}M spiperone. PRL increased basal DA release in a dose-dependent fashion with 10^{-5}M PRL being the most effective (232±51 vs 624±239 pg/mg; control vs PRL, $p<0.05$). Also, PRL attenuated AMPH-stimulated DA release in a dose-dependent manner with 10^{-5}M PRL being the most effective 1948±559 vs 878±273 pg.mg; control vs PRL, $p<0.05$). The effect of PRL was maintained in the absence of extracellular calcium. The addition of spiperone increased basal DA release similar to PRL (507±86 pg/mg, $p<0.05$ vs control). Also, spiperone in the presence of PRL blocked the PRL-induced attenuation of AMPH-stimulated DA release (2048±274 pg/mg). It is proposed that the effect of PRL on basal DA release is on D2 DA autoreceptors and that PRL interferes with AMPH-stimulated DA release via a spiperone site.

482.16

EFFECTS OF CG 3509, A TRH ANALOGUE, ON DOPAMINE RELEASE AS MEASURED BY *IN VIVO* VOLTAMMETRY. A. Gratton[1] and P.-P. Rompre[2]. Douglas Hosp. Res. Ctr, McGill Univ., Montreal, Canada, H4H 1R3[1] and Center for Studies in Behav. Neurobiol., Concordia Univ., Montreal, Canada, H3G 1M8[2].

Thyrotropin-releasing hormone (TRH) receptors are found in high densities in limbic structures. Some TRH receptors may be located on dopaminergic (DA) nerve terminals since 6-OHDA causes a decrease in the number of TRH receptors. Furthermore, TRH enhances locomotor activity when injected into the nuc. accumbens (NAC). Finally TRH and the TRH analogue, CG 3509, cause an increase in DA function. In the present study, the effects of CG 3509 on basal levels of DA as well as on K^+-evoked DA release were examined using *in vivo* electrochemistry. Chloral hydrate anesthetized rats were placed in a stereotaxic frame. A guide cannula was implanted into the lateral ventrical and a Nafion-coated, graphite/epoxy voltammetric electrode was lowered into the NAC. Electrochemical measures were obtained by applying to the voltammetric electrode at a rate of 1-5 Hz, a 0.5 V pulse in relation to a Ag/AgCl reference electrode. The current resulting from the oxidation of electroactive species was digitized and graphically displayed on a video monitor. Local K^+ stimulation was accomplished by pressure ejection of a 128 mM KCl solution from the tip of a pulled glass capillary cemented 200-250 um from the tip of the voltammetric electrode. Intraventricular infusions of CG 3509 (3-30 ug) had only negligeable effects on the basal electrochemical signal even at the highest dose. However, both the magnitude and the duration of K^+-evoked electrochemical signals were potentiated by CG 3509. The potentiating effect of CG 3509 was observed 45 min after injection and appeared to be maximal 90-120 min after injection. We are presently examining the effects of CG 3509 on DA release in other terminal fields. In conclusion the present data are consistent with the idea that TRH enhances dopaminergic function.

Supported by Medical Research Council of Canada grant MA-10735.

482.17

CHARACTERISTICS OF EXTRACELLULAR NOREPINEPHRINE : A MICRODIALYSIS STUDY IN GENETICALLY EPILEPSY-PRONE RATS. P.K. Mishra, R.L. Burger*, Q.S. Yan*, J.W. Dailey and P.C. Jobe. Dept. Basic Sci., Univ. of Illinois College of Medicine at Peoria, Peoria, IL 61656.

Abnormalities in the noradrenergic indices of the genetically epilepsy-prone rat (GEPR) have been well established as partial determinants of their seizure prone status. Intracerebral dialysis provides an excellent approach for studying functional aspects of the noradrenergic system and its role in seizure regulation. We have run a battery of standardization experiments in non-epileptic controls and GEPRs to test the reliability and feasibility of the microdialysis approach. Removable probes were used to perform microdialysis in awake and unrestrained animals. In each experiment, the probe was inserted in a stereotaxically implanted guide cannula. Dialysis was performed with artificial CSF at a rate of 1 ul/min and 20 min samples were analyzed by high-performance liquid chromatography coupled to electrochemical detection.

Repetitive insertion and removal of the probe over a 2 week period did not significantly alter extracellular levels of norepinephrine (NE). However, variations in NE levels were observed in an experiment in which a continuous sampling of the extracellular fluid was performed for 48 hours. These variations may be related to the activity of animal, time of day, or feeding time. In addition to the study of basal NE levels, we also observed alterations in its concentrations in response to physiological and pharmacological manipulations. High concentrations of potassium in the dialysis medium increased the amount of NE in the dialysate from striatum as well as from thalamus. An increase in extracellular concentrations of NE was also observed in thalamus upon treatment with its reuptake inhibitor, desipramine, as well as upon treatment with a presynaptic receptor inhibitor, yohimbine. These observations suggest that indices of extracellular norepinephrine in the microdialysate are reliable indicators of noradrenergic activity at the synaptic level.

482.18

THE RABBIT RETINA IN VITRO: A PHARMACOLOGICAL MODEL TO STUDY THE SYNAPTIC REGULATION OF DOPAMINE SYNTHESIS AND RELEASE. M. SCHORDERET AND S. OFORI*, Dept. of Pharmacol., CMU, Geneva, and Sch. of Pharm.,1005 Lausanne, Switzerland.

Rabbit retinas were used to investigate the synthesis, and/or release of endogenous dopamine (DA) by LC-EC (J.Neurochem., 47, 1199-1213, 1986). DA synthesis was assessed by measuring L-DOPA accumulation, in the presence of NSD-1015 (L-DOPA decarboxylase inhibitor); release by quantifying increased levels of DA in the incubation medium versus tissue DA. Thus, these conditions enabled us to dissociate DA synthesis from DA release. High K^+ (50mM) and forskolin (50μM) stimulated DA synthesis. Only the effects of high K^+ were reduced by (±)3-PPP (putative DA autoreceptor agonist). (±)3-PPP and quinpirole (DA_2 agonist) dose-dependently decreased DA synthesis. K^+ (50mM) caused a Ca^{++}-dependent release of DA. In addition, (±)amphetamine, reserpine and amfonelic acid induced a dose-dependent release of DA (independently of extracellular Ca^{++}). (±)amphetamine and reserpine release DA from two separate pools. These data indicate that studies using the rabbit retina in vitro, may lead to a better understanding of the regulation of dopaminergic activity at various synaptic sites.

482.19

EFFECT OF GLUTAMATE ON STRIATAL DOPAMINE RELEASE: IN VIVO DIALYSIS AND ELECTROCHEMICAL STUDIES. R.N. Adams, R.J. Gruen, B. Moghaddam, R.H. Roth and B.S. Bunney. Yale Univ., New Haven, CT 06511, and Univ. of Kansas, Lawrence, KS 66045.

We have examined the effect of local application of glutamate (GLU) on in vivo release of endogenous dopamine (DA) in the rat striatum (CP). In one group of experiments, dialysis probes were lowered bilaterally into the CP and recovered DA was assessed with HPLC-EC. In another group of experiments, ion-selective and Nafion coated carbon fiber microelectrodes were used to simultaneously monitor extracellular K^+ and DA levels. In the dialysis experiments, local infusion of 0.5μM GLU led to a significant decrease in basal DA release. Perfusion of 1μM, 50μM, 100μM, or 500μM GLU had no significant effect. Furthermore, local application of 500 μM GLU did not augment the K^+-stimulated release of DA. In the electrochemical studies, micropipette injections of approximately 100 nl of 0.1 mM and 1 mM GLU led to an increase in extracellular K^+ but not DA. Microinjection of 10 mM GLU, accompanied by a large increase in extracellular K^+ (20-50 mM), led to an increase in DA levels. It is likely that this GLU mediated increase in DA was an indirect effect caused by a massive depolarization or initiation of spreading depression since it was accompanied by a massive outflux of K^+. The dialysis and electrochemical data do not support the notion that GLU has an excitatory effect on the release of DA from nigrostriatal terminals. This work was supported in part by the following grants: MH14092; DA 05119; MH14276; and, MH25642.

482.20

INTRAVENOUS ADMINISTRATION OF THYROTROPIN-RELEASING HORMONE AND/OR TYROSINE CAN ALTER STRIATAL DOPAMINE RELEASE AS MEASURED IN VIVO BUT NOT IN VITRO. I.N.Acworth*, M.Kreutz*, H.Lehnert and R.J.Wurtman (Spon: N.Logothetis). Dept. of Brain and Cognitive Sciences, MIT, Cambridge, MA 02139.

In addition to its direct effect on anterior pituitary hormone secretion, previous evidence has suggested that thyrotropin-releasing hormone (TRH) may also alter central nervous neurotransmission, particularly that mediated by the monoamines. We have examined the effects of systemically administered TRH on striatal dopamine release in the anesthetized rat in vivo (using brain microdialysis) and in vitro (using striatal homogenates). Animals (microdialysis n = 7; homogenates n = 5) received TRH (10μg or 50μg i.v.) and/or tyrosine (as methyl-ester; 20mg/kg i.v.) or saline placebo. After probe implantation and when dopamine release had stabilized, the drugs were administered through the jugular catheter, and microdialysis samples (15min; 1.5μl/min) were collected for a further 150min. The same time-course was used for the in vitro study - animals were decapitated 150min after drug administration; the striata were removed and stored at -70°C until analysis. All samples were assayed for dopamine (DA), dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA) using HPLC-EC. TRH (10μg) increased DA levels in dialysate samples by 190% after two hours (levels of DOPAC and HVA were decreased by 15% and 20% respectively). Tyrosine alone increased DA release by 110%, while its coadministration with TRH (10μg) increased DA release by 450%. A higher dose of TRH (50μg) increased DA release by 750%. All these treatments failed to alter DA metabolism when studied using striatal homogenates. These findings suggest that TRH (or a metabolite) can enhance DA release, and that additional precursor amino acid (tyrosine) can potentiate DA release when its level becomes limiting due to sustained nigrostriatal firing. These data also suggest that increased DA release cannot always be predicted by in vitro homogenate studies.
(Supported in part by a grant from the Air Force Office of Scientific Research).

482.21

(−)-NALOXONE INCREASES CARBACHOL-EVOKED RELEASE OF CATECHOLAMINES, [MET]ENKEPHALIN, AND NEUROPEPTIDE Y FROM EX SITU-PERFUSED DOG ADRENAL. M. K. Dousa*, S. L. Chritton*, D. R. Roddy*, D. L. Lucas*, T. L. Yaksh and G. M. Tyce. Dept. of Physiology, Mayo Clinic, Rochester, MN and Dept. of Anesthesiology, University of San Diego, La Jolla, CA 92093.

(−)-Naloxone, a stereospecific opioid antagonist, was used to study the modulation of adrenomedullary secretion by endogenous opioid peptides. Experiments were carried out on retrogradely perfused ex situ dog adrenals using 2-min stimulations (S1, S2, S3) by $3x10^{-5}$M carbachol. Five min before S3, 10^{-5}M (−)-naloxone was added to perfusate for the rest of the experiment. In perfusates, collected before, during, and after each stimulation, catecholamines (CAs) were measured by HPLC with electrochemical detection and [Met]enkephalin (M-E) and neuropeptide Y (NPY) by specific radioimmunoassays. Introduction of 10^{-5}M (−)-naloxone into the medium did not change basal efflux of CAs and neuropeptides; however, net carbachol-evoked release of norepinephrine (NE), epinephrine (E), dopamine (DA), M-E, and NPY was increased to 138% for NE, 205% for E, 210% for DA, 216% for M-E, and 193% for NPY based on the release during S2 as 100%. No such increase occurred in the presence of (+)-naloxone. The above data showing stereospecificity of (−)-naloxone suggest a probable inhibitory effect of endogenous opioids on adrenomedullary secretion mediated by opioid receptors.

482.22

cis-UNSATURATED FATTY ACIDS STIMULATE CATECHOLAMINE SECRETION, TYROSINE HYDROXYLASE AND PROTEIN KINASE C IN BOVINE ADRENAL MEDULLARY CELLS. Y. Koda*, N. Yanagihara, A. Wada*, Y. Uezono*, H. Kobayashi* and F. Izumi. Depart. of Pharmacol. Univ. of Occupational and Environmental Health, Sch. of Med., Kitakyushu 807, Japan.

In digitonin-permeabilized bovine adrenal medullary cells, cis-unsaturated fatty acids (arachidonic acid and oleic acid) enhanced calcium-induced secretion of catecholamines and activation of tyrosine hydroxylase. On the other hand, trans-unsaturated fatty acid (elaidic acid) and saturated fatty acid (stearic acid) had no effect. Catecholamine secretion induced by arachidonic acid was abolished by the removal of ATP and magnesium from the incubation medium. Phorbol 12-myristate 13-acetate (PMA), an activator of protein kinase C, enhanced calcium-induced catecholamine secretion. The stimulatory effects of arachidonic acid and PMA were not additive. In soluble fraction of adrenal medullary cells, only cis-unsaturated fatty acids activated protein kinase C in a calcium-dependent manner.

These results suggest that cis-unsaturated fatty acids modulate catecholamine secretion and tyrosine hydroxylase activity by activation of protein kinase C in adrenal medullary cells.

482.23

PHOSPHORYLATION AND ACTIVATION OF TYROSINE HYDROXYLASE BY ELECTRICAL STIMULATION OF PERFUSED RAT ADRENALS. Ravindra K. Malhotra[1*], Arun R. Wakade[1] and John W. Haycock[2] (SPON: H. GOLDMAN). [1]Dept. Pharmacol., Wayne State Univ. Sch. Med., Detroit, MI 48201 and [2]Dept. Biochem. Molec. Biol., Louisiana State Univ. Med. Ctr., New Orleans, LA 70119

Electrical stimulation of the splanchnic nerve produces an exocytotic secretion of epinephrine and norepinephrine from rat adrenal medullary chromaffin cells. This effect is mediated by nicotinic, muscarinic and non-cholinergic receptors which are activated by acetylcholine (ACh) and VIP released from the splanchnic nerves. Due to the lack of high-affinity catecholamine (CA) uptake systems, rat adrenal chromaffin cells rely upon *de novo* biosynthesis to maintain endogenous CA stores. The present studies investigated the effects of electrical activation of the splanchnic nerve endings upon the phosphorylation and activity of tyrosine hydroxylase (TH), the initial and rate-limiting step in CA biosynthesis.

Isolated rat adrenal glands were perfused retrogradely with Krebs solution containing $^{32}P_i$ for 60 min and treated during the last 15 min of perfusion. Immediately after treatments, glands were frozen on dry ice. Samples were solubilized in SDS, and TH was immunoprecipitated and isolated by SDS-PAGE. ^{32}P incorporation into TH was quantified and normalized to ^{32}P incorporation into total cellular protein and to TH protein levels. Transmural electrical stimulation (10 Hz) increased ^{32}P incorporation into TH. Addition of ACh, nicotine, muscarine, or VIP also increased TH phosphorylation. After exhaustive digestion of the immunoprecipitated ^{32}P-TH, several phosphopeptides were separated by HPLC, indicating that the phosphorylation of TH was associated with multiple sites.

TH activity in desalted, high-speed supernatants was increased by electrical stimulation, cholinergic agonists or VIP. Cholinergic and VIP antagonists inhibited the activation. The role of the different phosphorylation sites in the activation of TH mediated by the different receptors is being investigated.

483.1

NORADRENALINE AXON TERMINALS IN ADULT RAT NEOCORTEX: SYNAPTIC INCIDENCE AND MICROENVIRONMENT. L. Descarries, P. Séguéla, K.C. Watkins* and M. Geffard. Centre de recherche en sciences neurologiques, Université de Montréal, Montréal, Canada H3C 3J7 and Laboratoire de Neuroimmunologie, I.B.C.N. CNRS, 33077 Bordeaux, France.

The relational characteristics of noradrenaline (NA) axonal varicosities from the upper layers of adult rat frontal, parietal and occipital cortex were investigated by PAP-electron microscope immunocytochemistry with an antiserum against NA-glutaraldehyde-protein conjugate. In serial as well as single thin sections, a large number of immunostained varicosities were scrutinized for the presence of a junctional membrane differentiation and the identity of juxtaposed elements. As a control, randomly chosen unlabeled varicosities from the same sections were similarly analyzed. The proportion of synaptic varicosities (incidence) was determined by linear transformation of the relationship between the observed frequency of junctional complexes and the number of thin sections available for examination. Using high or low stringency criteria for the definition of junctional complexes, the synaptic incidence of NA varicosities was thus evaluated at 17 and 26%, respectively, in sharp contrast with that of unlabeled varicosities (98%). The rare NA synapses were axo-dendritic, symmetrical and predominantly found on dendritic shafts versus spines. In each region, the microenvironment of NA varicosities also differed from that of the random population, with a greater number of axon varicosities and smaller number of dendritic spines. The neocortical NA innervation is therefore mostly non junctional (74-83%) and set in a particular milieu. These data also suggest that the global neuromodulatory roles assigned to the cortical NA input might be subserved by a comparable cellular distribution of NA transduction mechanisms throughout neocortex.

483.2

THE ADRENERGIC INNERVATION OF THE BED NUCLEUS OF THE STRIA TERMINALIS IN THE RAT BRAIN. T. Duong, R.S. Fisher, C.R. Houser and A.B. Scheibel. UCLA Departments of Psychiatry, Anatomy, and the Brain Research Institute, Los Angeles, CA 90024.

The adrenergic innervation of the bed nucleus of the stria terminalis (BNST) in the rat brain was studied by phenylethanolamine N-methyltransferase (PNMT) immunohistochemistry and retrograde tracer transport. PNMT-immunoreactive (PNMT-ir) somata were not observed in the BNST. PNMT-ir axons form a moderately dense plexus in the ventral, ventrolateral and lateroposterior subdivisions of the BNST. They are thin fibers with small (<1µm in diameter), widely spaced varicosities and they extend for long distances without branching. The terminal segments bear larger varicosities (1-3µm in diameter), arranged in pericellular basket formations. Retrograde tracer injections were performed in the BNST unilaterally and brainstem sections were processed for simultaneous demonstration of retrograde tracer and PNMT immunohistochemistry. Adrenergic neurons projecting to the BNST were observed bilaterally in the ventrolateral (C1) and the dorsomedial (C2) medullary groups, with a strong ipsilateral predominance. A small proportion of C3 adrenergic neurons located in and around the medial longitudinal fasciculus were also retrogradely labeled bilaterally. **The medullary adrenergic groups C1, C2, and C3 send mainly ipsilateral projections to the BNST. These projections are distributed densely to the ventral, ventrolateral, and lateroposterior portions of the BNST, and more diffusely to the rest of this forebrain nucleus.**

483.3

ULTRASTRUCTURAL BASIS FOR MODULATORY INTERACTIONS BETWEEN HIPPOCAMPAL AND DOPAMINERGIC AFFERENTS TO THE RAT NUCLEUS ACCUMBENS. S.R. Sesack and V.M. Pickel. Div. Neurobiology, Dept. Neurology, Cornell Univ. Med. College, NY, N.Y. 10021.

The mesolimbic dopamine system is known to modulate the excitatory influence of the hippocampus on nucleus accumbens neurons, providing one mechanism through which limbic afferents to the ventral striatum can regulate the motor output from the basal ganglia. We examined the ultrastructural basis for this interaction by combining anterograde degeneration resulting from fimbrial lesions of hippocampal afferents with peroxidase immunocytochemistry for tyrosine hydroxylase (TH). TH is a biochemical marker for catecholaminergic, principally dopaminergic terminals within the rat nucleus accumbens. Terminals of hippocampal fibers were also examined ultrastructurally following anterograde transport of wheat germ agglutinin-horseradish peroxidase (WGA-HRP) from the subiculum. Hippocampal afferents formed primarily asymmetric synaptic junctions with dendritic spines, many of which also received a symmetric contact from a TH-immunoreactive terminal. In addition, the membranes of degenerating and TH-immunoreactive terminals were often found in close apposition, although such contacts lacked obvious synaptic specializations. We conclude that hippocampal and dopaminergic afferents to the accumbens converge on common spiny neurons and may, additionally, interact at presynaptic sites. (Supported by grants NS08193 to S.R.S. and MH40342 to V.M.P.).

483.4

SOMATIC AND DENDRITIC BASIS FOR DOPAMINERGIC MODULATION IN THE VENTRAL TEGMENTAL AREA. V.E. Bayer and V.M. Pickel. Div. Neurobiology, Dept. Neurology and Neurosciences, Cornell Univ. Med. College. NY, NY 10021.

We examined the ultrastructure and synaptic associations of dopamine (DA)-containing neurons in the rat ventral tegmental area (VTA) using an antiserum against the catecholamine-synthesizing enzyme tyrosine hydroxylase (TH). Cells labeled for TH were visualized immunocytochemically by peroxidase-anti-peroxidase and immunoautoradiographic methods. These cells comprised a heterogeneous population differing in size, synaptic input and nuclear infolding. In addition to the usual subcellular organelles, some TH-labeled soma contained basal bodies of cilia and lamellar bodies. 55% (n=36) of soma and 15% (n=2,424) of dendrites labeled for TH were in apposition to other TH-labeled soma or dendrites. Of these TH-labeled dendrites, less than 2% received synaptic contacts from terminals also containing TH-immunoreactivity; while 49% had synaptic input from unlabeled terminals. From 2,282 observed unlabeled terminals, 5% showed dual synaptic input either onto 2 adjacent immunoreactive dendrites, 2 adjacent soma, or onto an adjacent labeled soma and dendrite. We conclude that dopaminergic neurons in the VTA (1) have few, if any, distinguishing cytological features, (2) show appositions and convergent input consistent with their synchronized firing and (3) are most likely modulated by DA release from dendrites and not DA-containing afferent axon terminals. Supported by grant NIMH-MH40342 to V.M.P.

483.5

COLOCALIZATION OF TYROSINE HYDROXYLASE AND G_o IN RAT STRIATAL SYNAPTOSOMES. M.E. Wolf, J.G. Granneman and G. Kapatos. Center for Cell Biology, Sinai Hospital of Detroit and Cellular and Clinical Neurobiology Program, Wayne State University, Detroit, MI 48235.

Dopaminergic striatal synaptosomes can be detected and isolated using a fluorescence-activated cell sorter (FACS) following permeabilization and labeling with a monoclonal antibody to tyrosine hydroxlase (TH) and fluorescent secondary antibody (Wolf and Kapatos, J. Neurosci. 9: 106, 1989). In the present study, double label FACS analysis using fluorescent secondary antibodies with different emission spectra was used to determine whether dopamine (DA) synaptosomes also contain G_o. G_o-containing striatal synaptosomes were detected with antibodies raised in rabbits against a synthetic peptide corresponding to a specific sequence of G_o and a fluorescein-labeled secondary antibody. Most particles in the synaptosomal fraction contained G_o (77.5 ± 3.9, n = 6). Specific labeling, defined with preimmune serum, was eliminated by preadsorption of anti-G_o antibodies with the peptide. TH-containing synaptosomes, detected with a monoclonal antibody to TH and a phycoerythrin-labeled secondary antibody, comprised 12.1 ± 0.6 % (n = 8) of total particles. Double label studies revealed that all TH-containing synaptosomes also contained G_o. This provides a basis for hypothesizing a signal transduction role for G_o in DA nerve terminals. Supported by MH 43657 (GK, MW), NS-08413 (MW) and DK 37006 (JG).

483.6

MAO-A AND PNMT CO-LOCALIZE IN HUMAN C-1 NEURONS: POSSIBLE ROLE OF DOPEGAL AS AN AUTOTOXIN IN ALZHEIMER"S DISEASE. D.L.Commins*,J.H.Haring,R.M.Denney,M.B.Mattammal*,H.D. Chung*,T.H.Joh,W.J.Burke Depts. of Anatomy and Neurology, St. Louis Univ., St. Louis VAMC, St. Louis, MO 63104.

We have recently found the MAO-A metabolite of epinephrine (Epi) 3,4 dihydroxyphenylglycolaldehyde (DOPEGAL) in human brain (Burke,W.J., Anal. Biochem., 1989, in press). To determine whether C-1 Epi neurons were a source of DOPEGAL, we performed immunocytochemistry on C-1 neurons using antibodies to MAO-A and phenylethanolamine N-methyltransferase (PNMT). Frozen slabs of C-1 were sectioned at 50 µm on a freezing microtome. Alternate sections were stained with either MAO-A or PNMT primary antibody diluted 1:1000. Secondary antibody conjugated to avidin-biotin-peroxidase complex together with diaminobenzidine were used to develop the stain. In other experiments to determine the potential toxicity of DOPEGAL, [^{3}H]norepinephrine was incubated with MAO to produce [^{3}H]DOPEGAL. The amount of binding of MAO-A product to DNA and tubulin was determined by comparing binding in reaction mixtures with and without MAO. Results show that in alternate sections C-1 neurons stain with MAO-A and PNMT suggesting that these enzymes co-localize in Epi neurons. Binding studies showed a binding of the MAO product of 1.8 nmoles/mg DNA and of 0.14 nmoles/mg tubulin. These results are consistent with the view that C-1 Epi neurons make a naturally occurring autotoxin. A role for this neurotoxin in Alzheimer's disease is being investigated.

483.7

COMPARISON OF DOPAMINE, NORADRENALINE AND TYROSINE HYDROXYLASE IMMUNOREACTIVITY IN THE RAT SPINAL CORD. J.G. WOLTERS*[1], H.W.M. STEINBUSCH[2] and J.G.J.M. BOL*[2]. (SPON: G.V. Lees) Depts. of Anatomy[1] and Pharmacology[2], Free University, Amsterdam.

Aim of the present study is to reveal the distributional differences of separate catecholamines in the rat spinal cord. Specific and sensitive antibodies to dopamine (DA), noradrenaline (NA) and tyrosine hydroxylase (TH) have been used. Roughly the distributions of DA-, TH- and NAi-immunoreactive (DAi, THi, NAi) varicose fibers are comparable. High density terminal fields are present in the dorsal part of the dorsal horn, in the lateral and central parts of the central grey, the intermediolateral cell column (IML) (T1-L1), and on motoneuronal cell clusters in the ventral horn. However, some conspicuous differences are observed: 1) In general DAi and THi terminal fibers are more finestructured and varicose than NAi fibers. 2) In the dorsal horn DAi fibers are densest in layers I and III, while layer II is nearly devoid; densest NAi fibers are found in layers I and II; a dense population of NAi but not DAi or THi cell bodies is present in layer II (L1-L2), and few THi neurons are seen in layer I (S1-S2). 3) THi cells are also present dorsal to the central canal (S1-S2) and in the lateral funiculus (S1) dorsolaterally to the sacral IML. These neurons do not stain for DA or NA. 4) In the ventral funiculus NAi and THi but not DAi descending fiber bundles are present. These results indicate a differential catecholaminergic input to the spinal cord, and the presence of intrinsic spinal NA (L1-L2) and possibly L-DOPA (S1-S2) neurons.

483.8

CHANGES IN CEREBRAL TYROSINE-HYDROXYLASE mRNA FOLLOWING ADRENALECTOMY: ANALYSIS BY QUANTITATIVE IN SITU HYBRIDIZATION. A.Berod*, M.Dussaillant*, A.Sarrieau*, B.Bloch* and W.Rostene. INSERM U55, 184 rue du Faub. St-Antoine 75012 Paris, Univ. Bordeaux II, 146 rue Leo Saignat 33076 Bordeaux FRANCE.

Numerous studies indicated that central catecholaminergic (CA) neurons are implicated as regulators of the hypothalamo-pituitary-adrenal axis during stress. Most of the CA neurons contain glucocorticoïd receptors. To assess the possible involvement of CA neurons in glucocorticoïd feedback action, changes in tyrosine-hydroxylase mRNA (TH-mRNA) in CA neurons (mainly in the locus coeruleus) of intact, long-term adrenalectomized and adrenalectomized, corticosterone-implanted rats. ^{35}S-labeled synthetic oligodeoxyribonucleotide probes were used to measure levels of TH-mRNA by means of quantitative in situ hybridization. Adrenalectomy for 8 days produced a significant increase of TH-mRNA (40%) in neurons of the locus coeruleus. This effect was totally prevented by subcutaneous implants of corticosterone in adrenalectomized rats. Subcutaneous implants of corticosterone or dexamethasone in intact rats were without effect on TH-mRNA expression in the locus coeruleus.

Our results suggest that corticosterone can inhibit TH gene expression in CA neurons and support the concept of corticosteroïd feedback action on CA neurons.

483.9

QUANTITATIVE AUTORADIOGRAPHY OF THE DOPAMINE UPTAKE COMPLEX IN THE CENTRAL NERVOUS SYSTEM.

E.K. Richfield and M. Herkenham. Neurology Deptartment, University of Rochester, Rochester, NY 14603 and Unit on Functional Neuroanatomy, NIMH, Bethesda, MD 20892

The reuptake of dopamine (DA), as a mechanism of neurotransmitter inactivation, has been of interest for a variety of reasons. Dysfunction of this complex results in neuropsychiatric disorders, forms the basis of therapy for these disorders and is the site of action of different psychostimulant drugs of abuse. Various drugs have been used in ligand binding experiments to study the anatomy and function of this complex. None have proven ideal, with each having different limitations. [^{3}H]-GBR 12935 is a ligand with a number of properties suggesting it might be a good candidate for studying this complex. We have developed a quantitative autoradiographic assay in rodent using [^{3}H]-GBR 12935 that provides several advantages over other assays, including increased sensitivity, specificity, signal-to-noise ratio and reduced cost.

Appropriate buffer studies demonstrated requisite incubation conditions, including a temperature of 2° C, 0.001% ascorbate, and 0.025% albumin. Kinetic studies ensured that equilibrium conditions were met and that binding was reversible. In saturation experiments, binding was saturable (B_{max} 6.50 pmol/mg protein, K_D was 2.1 nM, and n_H was 1.0). Competition experiments revealed binding to two sites, a nonspecific piperazine acceptor site and a specific DA uptake site. In the presence of trans-flupentixol, specific binding was to only the DA uptake complex as determined by the rank order of drugs known to bind to this site. Lesions of the substantia nigra resulted in a 90% decrease in this site in the striatum, whereas striatal kainate lesions did not reduce binding. The anatomical distribution described by this liagand is consistent with other pre- and post-synaptic markers of the DA system.

483.10

CATECHOLAMINE SYNTHESIZING ENZYMES IN HUMAN AND BOVINE ADRENAL TISSUE: IMMUNOHISTOCHEMISTRY, IN SITU AND NORTHERN BLOT ANALYSIS. T. Wessel, H. Baker, A. Towle, K.-T. Kim*, K. S. Kim* and T. Joh. Lab. of Mol. Neurobiology, Cornell Univ. Med. College, Burke Rehab. Center, White Plains, NY 10605.

Utilizing human adrenal tissue harvested immediately following kidney transplantation and fresh bovine adrenals, we have delineated the distribution of all four catecholamine (CA) synthesizing enzymes at the protein and mRNA levels. Rabbit antibodies produced against purified bovine enzymes tyrosine hydroxylase (TH), aromatic L- amino acid decarboxylase (AADC), dopamine-B-hydroxylase (DBH), and phenylethanolamine-N-methyltransferase (PNMT) exhibited a similar cellular distribution in the human and bovine adrenal medulla and revealed noradrenergic fibres in the outer adrenal cortex. Random-primed bovine cDNA probes were used for Northern blot and in situ analysis and efficiently hybridized with all four CA synthesizing enzyme mRNAs in both species. In situ hybridization revealed a comparable pattern for all four CA enzyme messages. A human DBH oligonucleotide displayed an overlapping hybridization pattern when compared to the random-primed bovine cDNA. Additional oligonucleotides were used to define differential exon expression for TH in the human adrenal; as expected exons 1 and 3 were found to be expressed strongly while no hybridization signal could be detected for exon 2. These results show that bovine cDNA and synthetic oligoprobes can be effectively used in conjunction with immunohistochemistry to study enzyme expression in human tissue. Supported by grant #'s NS 23103 and MH44043

483.11

IMMUNOCYTOCHEMICAL CO-LOCALIZATION OF MULTIPLE ANTIGENS IN CAROTID BODY TYPE I CELLS UTILIZING SERIAL SEMITHIN PLASTIC SECTIONS. L.J. Stensaas, Z.-Z. Wang, B. Dinger and S.J. Fidone. Dept. of Physiol., Univ. of Utah Sch. of Med., Salt Lake City, UT 84108

Preneural type I cells of the mammalian carotid body synthesize and release multiple neuroactive agents. Previous pre-embedding, double-labeling immunocytochemical studies indicated that pairs of these neuroactive agents commonly co-occur in the same cell (Soc. Neurosci. Abst., 46.6, 1988), but failed to reveal subsets of cells with unique patterns of co-occurrence. The present study examined the feasibility of utilizing post-embedding, avidin-biotin immunocytochemistry to co-localize substance P, met-enkephalin, tyrosine hydroxylase, dopamine beta hydroxylase, serotonin, and chromogranin in the same type I cells by means of thin (0.5 μm) serial plastic sections. Individual serial sections of osmicated tissue were deplasticised, treated with 1% periodic acid to remove osmium (Childs, Am. J. Anat., 175: 307, 1986), and immunostained with primary antibody in combination with Vector Elite ABC/HRP reagents. The results indicate that while four of the six markers could be co-localized within the same cell, they occurred in combinations which suggest the existence of functionally distinct subsets of cells. Supported by USPHS Grants NS07938 and NS12636.

484.1

NMDA RECEPTORS MAY BE INVOLVED IN THE TOXIC MECHANISM OF ACTION OF MDMA. KT Finnegan*, JJ Skratt*, I Irwin, and JW Langston. (Spon: J Tetrud) Institute for Medical Research and California Parkinson's Foundation, San Jose, CA 95128.

Despite a rapidly accumulating body of scientific literature on the biological effects of MDMA, little is known concerning its mechanism of toxicity. The NMDA receptor-gated, intracellular accumulation of calcium appears to be an important event underlying the neuronal damaging effects associated with ischemia/hypoxemia, hypoglycemia, and more recently the administration of methamphetamine (Sonsalla et al., 1989), a compound structurally related to MDMA. We therefore explored the role of NMDA receptors in the toxic actions of MDMA, using the systemically active non-competitive NMDA receptor antagonist, dextrorphan (DEX).

Groups of male Sprague-Dawley rats (N=6) were treated with either saline, MDMA (10mg/kg/dose), DEX (45 mg/kg/dose), or the combination of MDMA (10 mg/kg) and increasing doses of DEX (7.5-45.0 mg/kg/dose). DEX (given IP) and MDMA (given SC) were injected 5 times at 6 hr intervals; combined treatment groups received DEX 20 min before MDMA. Animals were killed 10 days later for assay of 5HT, DA, and their respective metabolites in the striatum, hippocampus, and cortex.

MDMA (10 mg/kg) alone consistently produced a 50 - 70% reduction in 5HT and 5HIAA in all areas. Striatal DA, DOPAC, and HVA were not affected. DEX (45 mg/kg) totally blocked the 5HT-depleting effects of MDMA in the striatum; lower doses provided proportionately less protection. In the hippocampus and cortex, significant protection was observed at only the 45 mg/kg dose of DEX. DEX alone produced inconsistent effects on 5HT, with small but significant increases or decreases noted in the striatum or cortex, respectively. These findings suggest that the NMDA receptor-calcium channel complex may be involved in the toxic mechanism of action of MDMA.

484.2

CELL TYPE-SPECIFIC TOXICITY OF EXCITATORY AMINO ACIDS FOR CULTURED MOUSE AND CHICK RETINAL NEURONS, D. Stenkamp, J. Coyle and R. Adler, Depts. of Neuroscience and Pharmacology, Johns Hopkins Univ. Sch. of Medicine, Baltimore, MD 21205.

We are investigating the mechanisms involved in the selective susceptibility of some retinal neurons to the toxic effects of excitatory amino acids. Using isolated retinal neurons and photoreceptors, grown in a chemically defined medium in the absence of glia, and with minimal intercellular contacts, this lab has shown that kainic acid (KA) spares photoreceptors but destroys neurons in a developmentally regulated, cell type-specific, dose-dependent manner. Pharmacological analysis now demonstrates that KA (EC50 = 200 μM) is two orders of magnitude more potent than glutamate. Quisqualate (QQ) receptor agonists affect a smaller proportion of the neurons, but elicit maximal responses at lower concentrations. These dose-response characteristics are similar for retinal cultures of embryonic chick and neonatal mouse. The findings suggest that the majority of these neurons are affected through KA-type receptors, while a smaller subpopulation may have QQ-type receptors. Under the culture conditions used NMDA is apparently not toxic at concentrations as high as 5mm. However, ongoing studies suggest that some cultured retinal neurons do express NMDA-type receptors. This apparent discrepancy is under investigation. Supported by an NSF graduate fellowship.

484.3

ONTOGENETIC DEVELOPMENT OF THE SENSITIVITY OF RAT STRIATAL NEURONS TO EXCITOTOXINS. C.M. Wray* and R.J. Boegman. (SPON: S. Ludwin). Dept. of Pharmacology and Toxicology, Queen's University, Kingston, Ontario. Canada, K7L 3N6.

We sought to establish the ontogenetic development of the neurotoxic actions of quinolinic acid (QUIN) and N-methyl-D-asparate (NMDA) on the three populations of striatal neurons using histochemical methods. Following an intrastriatal injection of QUIN (30 nmol) a full lesion did not develop until 23 post-natal days with the NADPH-diaphorase-positive neurons and enkephalinergic neurons, while 80% of the acetylcholinesterase (AChE)-positive neurons survived. An intrastriatal injection of NMDA (25 nmol) into an 11 post-natal day rat pup resulted in 32% cell survival of the diaphorase-positive neurons, which were observed to be swollen and without dendrites, 26% cell survival of the AChE-positive neurons and a full lesion of the enkephalinergic neurons. By 16 post-natal days, a full lesion had developed with no diaphorase-positive neurons present in the core injection area, while 30% of the AChE-positive neurons remained. Thus there is a differential ontogenetic development of the sensitivity of the diaphorase, AChE-positive and enkephalinergic neurons to the excitotoxins QUIN and NMDA. Supported by the Medical Research Council of Canada.

484.4

UPTAKE OF MPP+ BY N_2AB-1 NEUROBLASTOMA CELLS: RELATION TO MPP+ NEUROTOXICITY. S.J. Simmons and M.F.D. Notter. Environ. Health Sci. Ctr. and Dept. of Neurobio. and Anat., Univ. of Rochester Sch. of Med., Rochester, NY 14642.

A mouse adrenergic cell line(N_2AB-1) has been used to investigate the cell specific toxicity of 1-methyl-4-phenylpyridinium iodide(MPP+), the active metabolite of 1-methyl-4-phenyl-1,2,3,6 tetrahydropyridine(MPTP) in an in vitro model.

We have shown that mitotic cells are more sensitive to MPP+(3.4-340uM) toxicity than when cells are differentiated by prostaglandin E1 and dibutyryl cyclic AMP treatment for 3 days. Toxicity was assessed by morphology, cell number and protein incorporation.

To characterize this selective toxicity between different phenotypes, we have examined the MPP+ uptake properties of both mitotic and differentiated N_2AB-1 cells. Specific uptake/accumulation was measured after 30min exposure to [3H]-MPP+ ($3x10^{-8}$M).

CELL TYPE	fmoles/ug protein	fmoles/10^4cells
Mitotic	0.39 - 1.25	1.21 - 2.5
Differentiated	0.22 - 0.31	1.13 - 1.5

n=3wells/condition, range of 4 expts. Uptake was shown to be saturatable after 75min. Uptake of MPP+ was sodium-dependent and was inhibited by Benztropine, Mazindol, Desipramine and Fluoxetine. The decreased sensitivity of the differentiated N_2AB-1 cells could be due to the decreased accumulation of MPP+ within the cells or increased efflux. Experiments are ongoing to further characterize the selectivity seen in this in vitro model.

(Supported in part by NIEHS Pilot Grant ES01247 and NIH NS 25778.)

484.5

THE EFFECTS OF 1-METHYL,4-PHENYL,1,2,3,6-TETRAHYDROPYRIDINE (MPTP) ON THE BIOGENIC AMINES IN THE BRAIN OF THE CHICK EMBRYO. L.J. Cote, A.B. Naini, M. Vitale*, D. Biro*, V. Tennyson. Dept of Neurology and Anat & Cell Biol., Coll. of P&S, Columbia University, New York, N.Y. 10032.

The neurotoxin MPTP destroys dopamine (DA) neurons in primates, producing a permanent Parkinson-like syndrome. However,MPTP is far less toxic in rats and mice,suggesting that it may be metabolized differently in some species. For an easy to handle and inexpensive model to study the metabolism of MPTP we have studied its effects on the brain of the chick embryo. Newly fertilized eggs were maintained at 38°C in an egg incubator at about 50% humidity.On the 3rd day of gestation, a 0.5cm² window is made, 4ml of egg albumin is removed aseptically and the window is sealed with scotch tape. On the 13th day of gestation 40ul of a sterile solution of MPTP is applied as near as possible to a large blood vessel. On the 21st day of gestation (8 days after MPTP is added) the egg is opened, the entire chick brain is removed, rinsed in cold saline,weighed,homogenized in 0.3M PCA and centrifuged at 20,000Xg; for 20 min. The extract is assayed for DA, homovanillic acid(HVA),norepinephrine (NE),MHPG, serotonin (5-HT)and HIAA, using HPLC with EC detector.At 8X10-6 M MPTP in the egg,a 95% reduction in DA and NE occurs and a 70% decrease in 5-HT. Both HVA and MHPG are decreased by about 50%. At 8X10-7 M MPTP a 70% reduction in NE is observed but DA & 5HT are not significantly reduced.

The data indicates that MPTP affects all the biogenic amines(NE>DA>5-HT) in the chick brain. The chick embryo provides a useful model to study the neurotoxic properties of MPTP, and possibly other neurotoxins.

Supported by the Parkinson's Disease Foundation.

484.6

EFFECTS OF 2'-CH_3-MPTP AND MPTP ON DOPAMINERGIC SYSTEMS IN MICE : TYROSINE HYDROXYLASE AND GFAP IMMUNOCYTOCHEMICAL STUDIES. L. Gordon* and M. Gupta (SPON: B.K. Gupta). Dept. of Anatomical Sciences & Neurobiology, Univ. of Louisville Sch. Med., Louisville, KY 40292.

MPTP administration has led to a Parkinsonian state in humans and degeneration of the nigrostriatal system in monkeys and mice. In the present studies, effects of MPTP and an analog 2'-methyl MPTP (2'-CH_3-MPTP) were compared. Young adult male C57BL/6 mice were given multiple injections of 2'-CH_3-MPTP or MPTP over a two day period (total dose 45-60mg/kg i.p.). Three days later, all the control and treated mice were anesthetized and perfused with the fixative. Adjacent 40μm thick serial sections through the entire brain were stained immunocytochemically for tyrosine hydroxylase (TH) and Glial Acidic Fibrillary Protein (GFAP). The numbers of TH-positive neurons were quantitated in the substantia nigra (SN), the ventral tegmental area and A8 cell group, and GFAP-immunoreactive astrocytes in the striatum. The animals treated with 2'-CH_3-MPTP exhibited striking behavioral changes characterized by bradykinesia and stiff hind limbs, changes that were not so striking in animals treated with MPTP. There was virtually a complete loss of TH-positive neurons in the SN in 2'-CH_3-MPTP group compared to the MPTP group. Both groups showed extensive gliosis in the striatum compared to the controls. Studies of the effects on the ventral tegmental area and A8 cell group are currently in progress. These data demonstrate that MPTP produces extensive gliosis in the striatum and that 2'-CH_3-MPTP is much more toxic than MPTP. Supported by PHS grant R29 NS24291 to MG.

484.7

LONG-TERM SURVIVAL OF MPTP-TREATED MICE: TYROSINE HYROXYLASE AND GFAP IMMUNO-CYTOCHEMICAL STUDIES. M. Gupta. Dept. of Anatomical Sciences & Neurobiology, Univ. of Louisville Sch. Med., Louisville, KY 40292.

Previous studies from this laboratory have shown that dopaminergic neurons in the substantia nigra were reduced significantly after short term survival following MPTP treatment in mice. In the present studies, young adult male C57BL/6 mice were treated with MPTP over a two day period (total dose 60-90mg/kg i.p.). The animals were allowed to survive for 3 days, 2 months, and up to 4-6 months. After the appropriate survival times, control and MPTP treated mice were anesthetized and perfused intracardially with 4% paraformaldehyde and 1.5% sucrose in 0.1M PO_4 buffer. Serial 40µm thick sections were cut through the entire brain and adjacent sections were stained immunocytochemically for tyrosine hydroxylase (TH) and Glial Acidic Fibrillary Protein (GFAP). TH-positive neurons in the substantia nigra were quantitated in the control and MPTP- treated mice at different survival times. The number of GFAP stained astrocytes was quantitated in the striatum by sub-dividing it into a medial, lateral, dorsal and ventral compartments. The number of TH-immunoreactive neurons in the substantia nigra (SN) was reduced significantly three days after MPTP treatment. Furthermore, MPTP treatment produced extensive gliosis in the striatum demonstrated by the presence of numerous GFAP-stained astrocytes in these animals compared to the controls. The following will be discussed : (a) regeneration and plasticity of the dopamine neurons in the SN after long term survival following MPTP treatment and (b) astrocyte proliferation in the striatum with long-term survival. Supported by USPHS grant R29 NS24291.

484.8

SENSITIVITY OF VARIOUS TUMOR CELL LINES TO MPTP TOXICITY IN VITRO. G. Yorke, S. Davis*, M. Gupta and F.J. Roisen. Dept. of Anatomical Sciences & Neurobiology, Univ. of Louisville SCh. Med., Louisville, KY 40292.

Our earlier studies have shown that dopaminergic neurons in the substantia nigra are reduced significantly with MPTP treatment of mice. The present studies were carried out to investigate if (a) MPTP has a dose-dependent effect on the survival of PC12 cells in vitro, (b) the degree of differentiation of PC12 cells alters sensitivity to MPTP toxicity, (c) the cholinergic murine neuroblastoma line Neuro-2a and the rat glioma derived C6 line respond to MPTP toxicity. Undifferentiated PC12 cells were grown on collagen-coated surfaces in RPMI 1640 media supplemented with 10% horse serum and 5% FBS. Differentiated cells were cultured as above with the addition of 40ng/ml NGF. Neuro-2a cells were maintained in MEM and 10% FBS while C6 cells were cultured in Ham's F10 with 15% horse serum and 2.5% FBS . Cell survival was quantitated microscopically on coded cultures over a 4-6 day period. These studies demonstrate that MPTP has a dose-dependent effect on the survival of PC12 cells irrespective of their level of differentiation. Furthermore, the Neuro-2a and C6 exhibit similar cytotoxicity to MPTP. These results suggest that MPTP toxicity in vitro is not selective to catecholamine cells only since it also affects other cell types. Supported by USPHS grants NS24524 to FJR and NS24291 to MG.

484.9

PROTECTION OF MPTP TOXICITY WITH GANGLIOSIDES IN VIVO AND IN VITRO. F.J. Roisen, J. Schwarz* and M. Gupta. Dept. of Anatomical Sciences & Neurobiology, Univ. of Louisville Sch. Med., Louisville, KY 40292.

Gangliosides are relatively abundant components of the neuronal plasma membrane that stimulate neuronal differentiation in vitro as well as accelerate regeneration in the central nervous system. Ganglioside administration in vivo has been shown to enhance recovery after chemical or physical insult. We have shown that MPTP treatment in young adult mice leads to a decreased number of tyrosine hydroxylase (TH)-positive cell bodies in the substantia nigra (SN). Furthermore, we have also shown that MPTP has a dose-dependent effect on the survival of PC12 cells in vitro. The present studies were undertaken to determine if treatment of mice with gangliosides prior to MPTP injections directly into the brain has any effect on the dopaminergic neurons of the SN, and if pretreatment of PC12 cells with gangliosides and continued treatment with gangliosides in the presence of MPTP has any effect on MPTP toxicity in vitro. Young adult mice were anesthetized and injected stereotaxically with a mixture of bovine brain gangliosides (BBG) into each lateral ventricle (2µl /ventricle, conc. 200mg/ml). After 16-18 hr, half of the control and BBG injected animals were treated with MPTP (Gupta et al., Neurosci. Lett., 1986). Three days later, brains were processed for TH immunocytochemistry and TH neurons in the SN were quantitated. For in vitro studies, PC12 were grown in RPMI 1640 media supplemented with BBG and/or MPTP. Our results indicate that BBG pretreatment in vivo reduced MPTP toxicity in the SN. Treatment of PC12 cells with BBG also decreased MPTP's toxicity in vitro. Supported by grants NS 24524 to FJR and NS24291 to MG.

484.10

TRANSPLANTATION OF PRIMED AND NON-PRIMED PC12 CELLS IN THE STRIATUM OF MPTP-LESIONED MICE. X.L. Chen*, F.J. Roisen, C. Schwarz* and M. Gupta.(SPON: L.J. Embree). Dept. of Anatomical Sciences & Neurobiology, Univ. of Louisville Sch. Med., Louisville, KY 40292.

Numerous studies have shown the survival of grafted cells in different sites of the CNS in lesioned animals. We have shown previously that MPTP treatment in young adult C57BL/6 mice leads to a decreased number of tyrosine hydroxylase(TH)-positive neurons in the substantia nigra as well as dopamine levels in the striatum. In the present experiments (a) growth and survival of PC12 cells in MPTP-lesioned mice and (b) the effect of transplantation on the survival of TH-immunoreactive neurons in the host substantia nigra was investigated. Mice were given multiple injections of MPTP over a two day period (Gupta et al., Neurosci. Lett., 1986). Three days later, undifferentiated (NGF-naive) as well as NGF-primed PC12 cells (20,000 cells in 2µl medium/injection site) were transplanted stereotaxically at two sites into the striatum and allowed to survive for up-to two weeks. The animals were treated with Cyclosporin-A either prior to or after transplantation to suppress the immune system. Immunocytochemical staining for tyrosine hydroxylase was used to identify the transplanted PC12 cells in the striatum. The number of surviving TH-positive transplanted PC12 cells in the striatum for both groups as well as the number of TH-positive neurons in the host substantia nigra was determined. TH-immunoreactive PC12 cells were detected in the majority of the animals. The number of surviving cells ranged from below 100 to over a few thousand per animal. The effect of NGF priming on the survival of transplanted PC12 cells will be presented. Supported by grants NS24291 to MG and NS24524 to FJR.

484.11

EFFECT OF DEPRENYL ON MAO ACTIVITY AND MONOAMINE LEVELS IN DIFFERENT BRAIN REGIONS OF AGING MPTP-LESIONED MICE. H. Wiener* and M. Gupta (SPON: J. Chandler). Center for Neurochemistry, N.S. Kline Institute for Psychiatric Research, New York, NY 10035, and Dept. of Anatomical Sci. & Neurobiology, U of Louisville Sch. Med., Louisville, KY 40292.

Several studies have shown that deprenyl pretreatment prevents MPTP neurotoxicity. The present studies were undertaken to investigate if treatment of MPTP-lesioned mice with the MAO-B inhibitor, deprenyl, has any effect on monoamine oxidase(MAO)-B activity and monoamine levels in different brain regions. Male C57BL/6 mice at 18 months of age were treated with MPTP over a two day period (total dose 60-90mg/kg i.p.). Control animals received vehicle injections. Three days later, half of the control and treated mice were given deprenyl in drinking water (0.035mg/5ml) for 14-18 days. Fresh brains were taken out and several areas including striatum, olfactory tubercle and cortex were dissected, frozen on dry ice and analyzed for MAO activity as well as levels of monoamines and their metabolites. MPTP treatment alone ($p<0.01$) reduced the levels of dopamine in the striatum and olfactory tubercle and norepinephrine levels in the cortex. Treatment with deprenyl alone or MPTP followed by deprenyl reduced MAO-B activity in all three regions ($p<0.01$). These studies suggest that deprenyl treatment of MPTP-lesioned animals decreases MAO-B activity thereby preventing further deterioration of monoamine systems in the brain presumably by decreasing free radical formation, but has no effect on amelioration of MPTP toxicity. Supported by USPHS grant R29 NS24291 to MG.

485.1

PHARMACOLOGICAL PROFILE OF CGS 18102A, A PROPOSED ANXIOLYTIC WITH $5HT_{1A}$ AGONIST AND $5HT_2$ ANTAGONIST PROPERTIES. D.A. Bennett, M.A. Sills, M. Williams, R. Gerber, C.L. Amrick, W.C. Boyar, J.M. Liebman, R.A. Lovell* and A.J. Hutchison*. Research Department, CIBA-GEIGY Corporation, Summit, NJ 07901.

CGS 18102A, a hexahydrobenzopyranopyridine, inhibited ^{3}H-5HT (IC_{50}=14nM) and ^{3}H-8-OH-DPAT (IC_{50}=9nM) binding to the $5HT_{1A}$ receptor, and ^{3}H-ketanserin (IC_{50}=110nM) binding to the $5HT_2$ receptor. The compound was inactive at $5HT_{1B}$ sites (IC_{50}>10uM) and showed weak or no affinity for other neurotransmitter sites in vitro.

In vivo, CGS 18102A inhibited 5HTP accumulation in rat cortex (ED_{25}=2.7 mg/kg p.o.), indicating $5HT_{1A}$ agonist effects, but antagonized 5HTP-induced head twitch (ED_{50}=1.6 mg/kg i.p.) in mice, suggesting $5HT_2$ antagonist properties. The compound did not induce a 5HT syndrome in rats (30 mg/kg i.p.). CGS 18102A (1-17 mg/kg p.o.) was active in a Cook-Davidson model with increases in conflict responding of 29 to 156% above control baseline responding. These results are consistent with other $5HT_{1A}$ agonists in this model.

The combined $5HT_{1A}$ agonist and $5HT_2$ antagonist properties of CGS 18102A may suggest a compound with greater potential for anxiolytic effects than other compounds with only $5HT_{1A}$ agonist or $5HT_2$ antagonist properties.

485.2

ANXIOLYTIC ACTIVITY OF 5-HT1A AGONISTS AND ANTAGONISTS IN THE MOUSE. D. N. Johnson, R. Young, and S. Souders*. A. H. Robins Research Laboratories, Richmond, VA 23220.

With the reported activity of buspirone as an anxiolytic drug in man, new compounds with 5-HT1A agonist and antagonist properties have been tested for anxiolytic activity in various animal models. We have studied these compounds in the exploratory light/dark test (Costall et al., *J. Pharm. Pharmac.*, 40:302,1988). Buspirone (3.16-31.6 mg/kg IP) and ipsapirone (17.8-31.6 mg/kg IP) increased the time the mice spent in the lit area, from a vehicle control value of 31% to as much as 72% and 53%, respectively. The alleged pure 5-HT1A agonist, 8-OH-DPAT, was active in doses of 0.0001 to 3.16 mg/kg IP.

NAN-190, a putative 5-HT1A antagonist (Glennon et al., *Eur. J. Pharmac.* 154: 339, 1988), was inactive in increasing time in the lit area in doses up to 0.1 mg/kg IP. Higher doses resulted in a marked sedative effect (1.0-3.16 mg/kg IP) or lethality (10 mg/kg IP). Studies on rectal temperature in rats indicated that NAN-190 produced hypothermia at 3.16 to 17.8 mg/kg IP, but did not produce a concomitant Serotonin Syndrome. Furthermore, while NAN-190 did not potentiate the hypothermic effect of 8-OH-DPAT (0.25 mg/kg IP), 5.62 mg/kg IP antagonized the hypothermic effect of 8-OH-DPAT. Thus, although the hypothermic effect of NAN-190 was not due to a 5-HT1A agonist action, the blockade of 8-OH-DPAT hypothermia suggest that NAN-190 is a 5-HT1A antagonist.

The data presented here suggest that 5-HT1A agonists, but not 5-HT1A antagonists, may possess anxiolytic activity.

485.3

THE PHARMACOLOGICAL PROFILE OF 5-HT-1A RELATED ANXIOLYTICS: INTERACTION WITH 5-HT-1A, D-2 AND α-1 RECEPTORS. M. Nakamura*, H. Shimizu*, T. Tatsuno*, T. Tanaka*, Y. Kumasaka* and A. Hirose* (SPON: K. Scappaticci).Research Laboratories, Sumitomo Pharmaceuticals Co., Ltd., Osaka 554, Japan.

SM-3997 (3aα,4β,7β,7aα-hexahydro-2-(4-(4-(2-pyrimidinyl)-1-piperazinyl)-butyl)-4,7-methano-1H-isoindole-1,3(2H)-dione dihydrogen citrate) is a novel nonbenzodiazepine anxiolytic drug that produces anticonflict action via 5-HT-1A receptors in rats. To clarify the pharmacological properties of SM-3997 (SM), we performed biochemical, behavioral and electrophysiological studies comparing it with three other 5-HT-1A related anxiolytics, Buspirone (Bus), Ipsapirone (Ips) and Gepirone (Gep), and their common metabolite, 1-pyrimidinylpiperazine (1-PP).

SM and Bus showed higher anticonflict activity than Ips or Gep. Bus and Ips showed higher D-2 antagonist activity and α-1 antagonist activity, respectively, than the other anxiolytics. The 5-HT-1A agonist activity of Ips was lower than that of the other anxiolytics. 1-PP showed neither anticonflict activity nor 5-HT-1A agonist activity. These results suggested that the effect of SM on 5-HT-1A receptors is more selective than that of Bus or Ips, and is stronger than that of Gep. It was also suggested that 1-PP does not participate in the anticonflict action of these anxiolytics.

485.4

REGIONAL DISTRIBUTION AND PHARMACOLOGICAL PROPERTIES OF [3H]SM-3997 BINDING SITES IN THE RAT BRAIN. H.Shimizu*, H.Tanaka*, T.Tatsuno*, A.Hirose*, Y.Kumasaka* and M.Nakamura*(SPON:G.Ryan). Research Laboratories, Sumitomo Pharmaceuticals Co., Ltd., Osaka 554, Japan.

SM-3997(3aα,4β,7β,7aα-hexahydro-2-(4-(4-(2-pyrimidinyl)-1-piperazinyl)-butyl)-4,7-methano-1H-isoindole-1,3(2H)-dione dihydrogen citrate) is a clinically effective anxiolytic that does not interact with the benzodiazepine-GABA receptor complex, but is suggested to have 5-HT-1A agonistic properties. The present study was conducted to determine the autoradiographic localization and characterization of [3H]SM-3997 binding sites in the rat brain.

Binding sites for [3H]SM-3997 were concentrated in the areas of 5-HT neuronal cell bodies and synaptic terminals. Very high densities of these sites were found in the dorsal raphe, dentate gyrus, CA1 field of Ammon's horn, lateral septum and the entorhinal cortex. In contrast, they were sparse in the substantia nigra and cerebellum. The distribution pattern of [3H]SM-3997 binding sites was nearly equal to that of [3H]8-OH-DPAT binding sites. In any brain structure we determined, [3H]SM-3997 binding was selectively inhibited by the ligands which have high affinity for 5-HT-1A receptors, such as 5-HT, 8-OH-DPAT, pindolol and buspirone.

These findings suggested that the novel anxiolytic SM-3997 selectively interacted with 5-HT-1A receptors in the rat central nervous system.

485.5

POTENTIAL ANXIOGENIC COMPONENT OF THE FORCED SWIM TEST. S.A. Welner, D. Ramdoyal* and B.E. Suranyi-Cadotte. Douglas Hospital Research Centre, McGill Univ., Dept. Psychiatry, Montreal, Quebec, Canada H4H 1R3.

The forced swim test has been used as an animal model to screen antidepressant drug activity; single injections of certain antidepressant drugs will reverse the learned helplessness effect evident in untreated animals. We report here that, additionally, there is an anxiogenic component to this test; treatment of male Long-Evans rats for 3 or 21 days prior to the training session of the forced swim test with the anxiolytic drug, diazepam (2 mg/kg; i.p.), resulted in significantly decreased immobility time in the forced swim retest when compared to a vehicle-treated control group. Neither 3 nor 21 day diazepam-treated groups differed on the retest from groups of rats treated for the same time periods with the classical antidepressant drug amitriptyline (10 mg/kg; i.p.). Interestingly, on the training session day, 3 day treatment with diazepam alone decreased the immobility latency during the first 5 minutes of the training session, whereas 21 day treatment of both diazepam or amitriptyline resulted in decreased immobility latencies during this first 5 minute period. This study suggests that the anxiogenic component of the forced swim test is significant and that biochemical changes attributed to effects in depression with this model should be made with caution.

485.6

NEUROPHARMACOLOGY OF 5-HT1A AGONISTS: EFFECTS ON FIRING RATES OF NOREPINEPHRINE CELLS MORE RELATED TO EFFECTS ON DOPAMINE CELLS THAN TO EFFECTS ON 5-HT CELLS. J.T.Lum and M.F.Piercey, CNS Research, The Upjohn Company, Kalamazoo, MI 49001.

8-OH-DPAT, buspirone, gepirone, ipsapirone, and U-67413B were compared for their effects on firing rates of rat dorsal and median raphe 5-HT neurons (DR and MnR), SNPC dopamine (DA) neurons, and locus coeruleus norepinephrine (NE) neurons. Ipsapirone depressed 5-HT neurons in MnR and DR with ED50's of 11 and 16 ug/kg i.v., respectively, and antagonized amphetamine-induced depressions of DA cells (ED50=375 ug/kg). 8-OH-DPAT, buspirone, and gepirone had similar profiles. Potencies (ug/kg) for 8-OH-DPAT were 1.6 (DR), 2.5 (MnR), and 612 (amphet. antag. in SNPC); for gepirone they were 7.4 (DR), 32.5 (MnR), and 218 (amphet. antag. in SNPC), while for buspirone they were 15 (DR) and 11 (amphet. antag.). U-67413B depressed 5-HT and DA cells with ED50's of 50 ug/kg (Piercey et al., this meeting). 8-OH-DPAT, buspirone, ipsapirone, and gepirone stimulated NE neurons with potencies similar to DA antagonism, but U-67413B depressed NE neurons with a potency similar to that for DA agonism. It is concluded that effects of 5-HT1A agonists on NE cells are more related to effects on DA cells rather than to effects on 5-HT cells.

485.7

U-67413B, A DIHYDROPHENALENE 5-HT1A AGONIST WITH WEAK DOPAMINE AGONIST PROPERTIES. J.Szmuszkovicz, R.A.Lahti, P.F.VonVoigtlander, A.H.Tang, J.S.Althaus, J.T.Lum, W.E.Hoffmann, D.L.Evans, S.R.Franklin, R.A.Code*, and M.F.Piercey (spon. M.Camacho-Ochoa), CNS Research, The Upjohn Company, Kalamazoo, MI 49001.

U-67413B (1H-phenalen-4-ol,2-(dipropylamine)-2,3-dihydro-monohydrobromide), was found to be a 5-HT1A agonist with dopaminergic agonist properties. Other standard 5-HT1A agonists, in contrast, are dopamine antagonists with varying selectivity (J.T.Lum and M.F.Piercey, this meeting). U-67413B displaced 3H-8-OH-DPAT from 5-HT1A binding sites with a potencey of 22 nM. Behaviorally, 10-30 mg/kg s.c. displayed the typical 5-HT1A syndrome in rats (forepaw treading, flattening of posture) with no evidence of dopaminergic activity. Hypothermia in mice had an ED50 of 2.3 mg/kg s.c., less than that for buspirone (3.1), but more than that for gepirone (1.3), ipsapirone (1.3), and 8-OHDPAT (0.13). Similarly, U-67413B was less potent than standard agonists in depressing 5-HT neuron firing rates in rat dorsal raphe (ED50=50 ug/kg i.v. vs 1.6-15 ug/kg). On dopamine cells, U-67413B partially depressed firing rates (ED50=50 ug/kg). Using HPLC, 10 mg/kg s.c. U-67413B decreased 5-HTP, 5-HT, 5-HTP, 5-HIAA, DOPA, DOPAC, and HVA. Diazepam and U-67413B displayed anxiolytic activity on the mouse 4-plate test, but buspirone did not.

485.8

BUSPIRONE: EFFECTS ON SEROTONIN/NOREPINEPHRINE NERVE IMPULSE FREQUENCY AND RELEASE MECHANISMS. P.A.Broderick and F.T.Phelan*, J.T.Lum, W.E.Hoffmann, and M.F.Piercey. Pharmacology Dept., CUNY Medical School, Convent Ave. and 138th St., NY, NY 10031, and CNS Research, The Upjohn Company, Kalamazoo, MI 49001.

Both norepinephrine (NE) and 5-HT systems have been implicated as being anxiogenic. Both are depressed by benzodiazepine anxiolytics. We have used microelectrode recordings of nerve cell impulse frequency and in vivo voltammetry recordings of hippocampal 5-HT (Neuropeptides 10:369, 1987) and NE (Neurosci. Lett. 95:275, 1988) release to evaluate the effects of the 5-HT1A anxiolytic buspirone on rat 5-HT and NE neurons. As previously reported, (Eur. J. Pharmacol. 149:9, 1988), buspirone depressed 5-HT neuron firing rates in dorsal raphe with a potency of 15 ug/kg i.v. It increased NE neuron firing rates in locus coeruleus with a dose-response curve virtually congruent with that for 5-HT neuron depression. In hippocampus, 1 mg/kg s.c. buspirone decreased 5-HT release moderately, but dramatically decreased NE release. It is concluded that, like benzodiazepines, buspirone depresses both NE and 5-HT systems and that the increase in NE neuron firing rates is a response to negative feedback control.

485.9

ANTAGONISM OF 5HT CELL BODY AUTORECEPTORS (5HT1A SUBTYPE) BY THE DOPAMINE AUTORECEPTOR ANTAGONIST (+)-AJ 76. M.F. Piercey, J.T.Lum and W.E.Hoffmann. CNS Research, The Upjohn Company, Kalamazoo, MI. 49001.

The dopamine autoreceptor antagonist (+)-AJ 76 (Svensson et al., 1986, Arch. Pharmacol., 334:234 and Hoffmann et al., 1988, Neurosci. Abs. 14:524) was evaluated for its effects on firing rates of rat 5-HT neurons in dorsal raphe. (+)-AJ 76 weakly depressed firing rates of 5-HT neurons. Starting with a threshold dose of 100 ug/kg i.v., there was a shallow dose-response curve which peaked at about 50% depression with 3000 ug/kg of drug. In animals pretreated with 10 mg/kg (+)-AJ 76, the ED50 for the potent 5-HT1A agonist 8-OH DPAT was 48 ug/kg compared to only 1.3 ug/kg in untreated animals. It is concluded that (+)-AJ 76 is a partial agonist at the 5-HT1A autoreceptor. (+)-AJ 76 also antagonized the hypothermic effects of 5-HT1A in mice, an effect probably induced by postsynaptic 5-HT1A receptors. A dose of 30 mg/kg s.c. shifted the ED50 for 8-OH DPAT from 0.23 mg/kg to 1.73 mg/kg, but did not at all affect body temperature itself. (+)-AJ 76 had little effect on norepinephrine cell firing rates. It is concluded that (+)-AJ 76 is a 5-HT1A receptor antagonist with weak agonist intrinsic activity that is observable only in the presence of large numbers of spare receptors (e.g. dorsal raphe).

485.10

2-DEOXYGLUCOSE AUTORADIOGRAPHY PINPOINTS SEROTONIN NEURON DEPRESSION AS A COMMON MODE OF ACTION FOR 5-HT1A AND BENZODIAZEPINE-TYPE ANXIOLYTICS. W.E.Hoffmann and M.F. Piercey, CNS Research, The Upjohn Company, Kalamazoo, MI 49001 USA.

2-Deoxyglucose autoradiography in rats was used to compare regional metabolic effects of 5-HT1A agonist anxiolytics (buspirone, U-67413B), to those for alprazolam, a benzodiazepine anxiolytic, and FG 7142, an anxiogenic benzodiazepine inverse agonist. U-67413B was chosen because, unlike buspirone, it totally lacks dopamine antagonism and does not excite locus coeruleus cells. Buspirone, 3 or 10 mg/kg i.p., and U-67413B, 10 mg/kg i.p., depressed metabolism in 35 of 71 brain areas. Thirty-five regions were depressed by alprazolam, 3 mg/kg.i.p. and/or stimulated by FG 7412, 10 or 20 mg/kg, i.p. Regions affected by each 5-HT1A agonist were highly correlated with each other, but not with those affected by the benzodiazepine receptor ligands. 5-HT1A agonists depressed metabolism in 5-HT cell body (dorsal and median raphe, interpeduncular n.) and projection areas. The hippocampus and wide areas of the cerebral cortex (cingulate, entorhinal, parietal, visual and motor) were all depressed. All four drugs affected 5-HT cell body areas (interpeduncular n., median raphe) and the cingulate and retrosplenial cerebral cortices. The results are consistent, with 5-HT neuron depression as a common site for benzodiazepine and non-benzodiazepine anxiolytics.

485.11

EFFECT OF THREE BENZODIAZEPINES ON MONGOLIAN GERBIL ACTIVITY. K. C. WOLFF*, A. E. HARRIMAN*, AND S. SANGIAH. Lab of Comparative Psychology, OK. State Univ. Dept. of Psych., Stillwater, OK 74078.

Four male and four female gerbils were deprived of food 24 hrs before intraperitoneal injections of Normal Saline 0.1 cc, 0.3 cc, 0.6 cc; Flurazepam 30 mg/kg, 45 mg/kg, 60 mg/kg; Temazepam 0.75 mg/kg, 1.5 mg/kg, 3.0 mg/kg; and Triazolam 0.0125 mg/kg, 0.025 mg/kg, 0.05 mg/kg. In addition, sham injections were also used. The administration of drug and dosage was conducted and analyzed using a factorial repeated measures design. Results suggest that all dosage levels of Flurazepam and Temazepam increase bar pressing as measured in the Skinner Box using a schedule of continuous reinforcement. On the other hand, all dosage levels of Triazolam decreased the rate of bar pressing.

The second part of the study ivolved twelve naive male gerbils, each receiving the same drug and dosage level used in the first part of the experiment. The analysis was also a factorial repeated measures design. The animals were tested using an activity wheel and then they were placed in an open field situation. Results indicated that those gerbils receiving Flurazepam and Temazepam had an increase in motor activity as well as grooming, marking, rearing, and leaping behavior. The opposite was found for those gerbils receiving Triazolam. We also found an increase in seizure activity among those gerbils receiving 45 mg/kg of Flurazepam and 1.5 mg/kg of Temazepam.

485.12

THE LATE EXPLORATORY TEST FOR HYPNOTICS IN MICE. P.J.K.D.Schreur, N.F.Nichols, J.W.Francis*, and L.H.Jensen. CNS Research, The Upjohn Company, Kalamazoo, MI 49001, and Ferrosan CNS Division, Soeborg, Denmark.

The late exploratory test was developed in mice to predict activity of new hypnotics in humans.

Pairs of male CF-1 mice were placed into 8" x 8" Omnitech Digiscan chambers under room light immediately after i.p. injection of triazolam, flurazepam, diazepam, zopiclone, zolpidem, diphenhydramine, Na pentobarbital, Na phenobarbital, RO 16-6028, chloral hydrate (oral), or vehicle. After 15 min, when the mice were partially habituated to the chambers, locomotor activity (total distance) was recorded automatically for the next 5 min. By linear regression for these 10 standards, the minimum effective dose (m.e.d.) correlated with the human oral hypnotic dose ($r = 0.820$, $p = 0.004$).

In conclusion, the late exploratory test predicts human hypnotic activity of compounds.

485.13

THE DARK MOTILITY TEST FOR HYPNOTICS IN MICE. N.F.Nichols, P.J.K.D.Schreur, J.W.Francis*, and L.H.Jensen. CNS Research, The Upjohn Company, Kalamazoo, MI 49001, and Ferrosan CNS Division, Soeborg, Denmark.

The dark motility test in mice was developed to predict human hypnotic activity for new compounds.

Standard hypnotics decreased habituated (non-exploratory), open-field behavior of female CF-1 mice tested in Omnitech Digiscan monitors during their most active part of the day (the dark phase). At least one hr after the lights were turned off, groups of 4 mice were placed into the 16" x 16" monitors. One hr later, the mice were injected intraperitoneally (i.p.) with triazolam, flurazepam, diazepam, zopiclone, zolpidem, diphenhydramine, Na pentobarbital, Na phenobarbital, chloral hydrate, or vehicle. Thirty min after injection, locomotor activity was measured for two 30-min periods. The minimum effective dose (m.e.d.) was the lowest dose which decreased total distance in either 30-min period. By linear regression for these 9 standards, the m.e.d. correlated with the human oral hypnotic dose ($r = 0.818$, $p = 0.007$).

In conclusion, the dark motility test predicts human hypnotic activity of compounds.

485.14

BUSPIRONE, GEPIRONE, IPSAPIRONE, AND 8-OH-DPAT: COMPARISON OF TWO MOUSE AGGRESSION MODELS. J.A.Oostveen* and P.J.K.D.Schreur, CNS Research, The Upjohn Company, Kalamazoo, MI 49001.

Four putative 5-HT1A anxiolytics were tested for their effects on aggression elicited by footshock or by chronic isolation. In the footshock model, pairs of male CF-1 mice (28-30 g) were confined in a small space and shocked via the floor grid (20 sec maximum) until dominance was established. Pairs of dominant mice were injected with drug i.p. 30 min before receiving intermittent shocks (maximum = 15 shocks). Mice were separated as soon as fighting began and the number of shocks was recorded. For isolation aggression, male CF-1 mice were housed singly or in groups of 4 for at least one month. An isolated "resident" mouse was injected i.p. with drug 30 min before an untreated, group-housed "intruder" was introduced. The intruder was removed as soon as fighting began and the number of seconds was recorded.

Buspirone HCl was active at 3-30 mg/kg in the isolation model but inactive in footshock to 30 mg/kg. For isolation and footshock models, respectively, 8-OH-DPAT HBr (8-hydroxy-dipropylaminotetralin HBr) was active at 1-10 mg/kg and 3-10 mg/kg, gepirone HCl at 3-30 mg/kg and 10-30 mg/kg, and ipsapirone HCl at 10-30 mg/kg and 30 mg/kg. Isolation-induced aggression is a more sensitive test than footshock-induced aggression for these 5-HT1A anxiolytics.

485.15

IS DEXTROMETHORPHAN A SIGMA ANTAGONIST? INTERACTIONS WITH (+)SKF10047. F. C. Tortella and L. Robles*, Neuropharmacology Br., Div. of Neuropsychiatry, Walter Reed Army Institute of Research, Washington, DC 20307-5100.

The non-opioid antitussive Dextromethorphan (DM) and the sigma ligand (+)SKF10047 [(+)SKF] appear to share a common binding site, yet (+)SKF is psychotomimetic and DM is not. Using an in vivo EEG and behavioral model, we have tested the possibility that DM is a sigma antagonist. In rats treated with DM (15 mg/kg, i.v.) or (+)SKF (10 mg/kg, i.v.), distinct EEG and behavioral profiles emerge. DM produces a brief period of sedation followed by ataxia. The significant EEG correlates are an increase in spectral power in the 1-5 and 7.5-10 Hz range and decreases in complexity and edge frequency. In contrast, (+)SKF produces a complex continuum of EEG and behavioral changes characterized by behavioral immobility, head-weaving and ataxic locomotor activity. A "dual" peak EEG spectra predominates with mean peak frequencies of 2.1 and 5.5 Hz. (+)SKF also causes significant decreases in the mobility, complexity, mean frequency and edge frequency of the EEG. Pretreatment with DM antagonizes these "psychotomimetic" effects of (+)SKF. The head-weave and ataxic locomotor activity were markedly attenuated, and the (+)SKF-induced increase in EEG spectral power in the 5-7.5 Hz band, and decrease in mobility, complexity and mean frequency, were also antagonized.

While DM and (+)SKF may share a common binding site, it appears that in doing so DM may act as a sigma antagonist, capable of attenuating the psychotomimetic activity of (+)SKF.

485.16

ANTAGONISM OF A MODEL SIGMA SYNDROME. Edgar T. Iwamoto. Dept. of Pharmacology, College of Medicine, Univ. of Kentucky, Lexington, KY 40536

We have presented evidence for a drug-induced activation of central sigma systems in rats (Life Sci. 44:1547, 1989) in which motor behavior is characterized by an initial 20 min period of retropulsion (RETRO) and sideways-circling (SIDE) followed by 90 to 100 min of forward locomotion (LOCO). The syndrome is initiated by a subcutaneous (SC) injection of (+)-butaclamol ((+)-BUT), 1.6 mg/kg, given 30 min before 10 mg/kg SC of (-)-N-allylnormetazocine ((-)-NAN) in Sprague-Dawley male rats which had been administered four daily injections of 10 mg/kg of (-)-NAN SC. The syndrome is antagonized by (+)-BMY 14802, rimcazole and haloperidol but not by naltrexone, MR 2266, R(+)-SCH-23390 or S(-)-sulpiride. The data suggest that the manifestation of the (+)-BUT/(-)-NAN-induced syndrome depends upon intact neurotransmission at central sigma sites.

Adult male rats were sensitized to (-)-NAN with four daily 10 mg/kg SC injections. On Day 5, animals were administered 1.6 mg/kg SC of (+)-BUT, and 10 min later, were injected with various doses of putative sigma receptor ligands followed 20 min later by a fifth injection of (-)-NAN. Both (+)- and (-)-BMY 14802 decreased RETRO, SIDE and LOCO at doses of 2.5 to 20 mg/kg SC; the 20 mg/kg dose of either isomer inhibited RETRO and SIDE and decreased LOCO by 60 to 80%. Fluphenazine, 0.033, 0.1 and 0.33 mg/kg SC, induced dose-related inhibition of the (+)-BUT/(-)-NAN-induced RETRO and SIDE with complete inhibition at the 0.33 mg/kg dose; LOCO was unaltered by these doses. In contrast, doses of 0.033, 0.1 and 0.33 mg/kg SC of spiperone did not alter the RETRO or SIDE of the sigma syndrome; LOCO was stimulated by the 0.33 mg/kg pretreatment of spiperone. Since fluphenazine binds with high affinity and spiperone has weak affinity for the sigma receptor, the data support the contention that the model sigma syndrome represents a drug-induced activation of endogenous sigma systems. The data further suggest that the (+)-BUT/(-)-NAN-induced signs of RETRO and SIDE in (-)-NAN-sensitized rats are more appropriate endpoints of sigma activation than the LOCO endpoint.

485.17

SERTRALINE: A POTENT INHIBITOR OF (+)[^{3}H]3-PPP BINDING TO BRAIN SIGMA (σ) RECEPTORS. B. K. Koe, L. A. Lebel*, C. A. Burkhart* and A. W. Schmidt. Central Research Division, Pfizer Inc., Groton, Connecticut 06340.

Sigma receptors in brain are defined as the high affinity, haloperidol-sensitive binding sites labeled by (+)[^{3}H]N-allylnormetazocine (NAN; SK&F 10,047). (+)[^{3}H]3-(3-Hydroxyphenyl)-N-n-propylpiperidine (3-PPP) and [^{3}H]di-o-tolylguanidine (DTG) are also radioligands for σ binding sites. Affinity for σ receptors has been invoked to account for potential antipsychotic activity (as predicted by animal behavioral tests) of agents that are not dopamine receptor antagonists; e.g., rimcazole (Ferris et al., 1986) and BMY 14802 (Taylor and Dekleva, 1987).

Sertraline, a new antidepressant and selective 5-HT uptake blocker (Koe et al., 1983), was found to be a potent inhibitor of (+)[^{3}H]3-PPP binding to rat brain membranes (IC_{50} 7 nM). Sertraline's high affinity for σ receptors was dependent on its conformation as well as on the Cl atoms in its pendant phenyl ring. Efficacy of binding to σ receptors in brain in vivo was determined by comparing the labeling of brain σ sites of control and drug-treated mice after i.v. injection of (+)[^{3}H]3-PPP (Koe et al., 1989). Affinity for σ receptors in vivo of several 5-HT blockers and σ ligands decreased as follows: sertraline > norsertraline > DTG, fluoxetine, femoxetine > BMY 14802 > paroxetine, rimcazole, (-)-butaclamol. The in vivo potency of sertraline for inhibiting (+)[^{3}H]3-PPP binding to σ receptors in mouse brain was the same as that for blocking 5-HT uptake in rat brain (ID_{50} 0.7 μmol/kg i.p.).

485.18

STEREOSELECTIVE RECEPTOR BINDING AND BIOLOGICAL ACTION OF THE POTENTIAL SIGMA ANTIPSYCHOTIC BMY 14802. Duncan P. Taylor, Susan H. Behling*, Jennifer Dekleva*, and Michael S. Eison. CNS Biology, Bristol-Myers Company, P.O. Box 5100, Wallingford, CT 06492-7660.

BMY 14802 has been identified as a potential antipsychotic agent in behavioral testing (Soc. Neurosci. Abstr. 11: 114, 1985). We have shown that this agent selectively and competitively inhibits sigma binding but not PCP or dopamine binding (op. cit. 14: 371, 1988). BMY 14802 stereoselectively inhibits sigma binding, and we have explored the biological actions of its enantiomers in several behavioral systems. The more potent sigma binding inhibitor (+)BMY 14802 is also more potent than the levorotatory enantiomer in the inhibition of the conditioned avoidance response and in the reversal of catalepsy induced by trifluoperazine. The less potent sigma binding inhibitor (-)BMY 14802 is more potent in blocking apomorphine-induced stereotypy. Further results from other tests with these enantiomers will be presented.

Test	(+)BMY 14802	(-)BMY 14802
[^{3}H]DTG Binding	32	140
Conditioned Avoidance	22	39 (30-50)
Apomorphine Stereotypy	44 (38-50)	25 (17-37)
Catalepsy Reversal	11 (7-19)	38 (27-34)

Data are K_I (nM) or ED_{50} (mg/kg, p.o.) with 95% fiducial limits in parenthesis, for tests in rats.

485.19

POST-JUNCTIONAL INHIBITION BY SIGMA AND PCP LIGANDS OF RAT TAIL ARTERY CONTRACTILE RESPONSES. T. Massamiri* and S.P.Duckles. (SPON: J. ONO) Department of Pharmacology, College of Medicine, University of California, Irvine, CA 92717.

(+)3PPP (3-[3-hydroxyphenyl]-N-(1-propyl)-piperidine) acts on both dopamine and sigma receptors in the perfused rat tail artery in vitro, confirming previous reports. We also demonstrate an additional mechanism of action of (+)3PPP; (+)SKF 10047, (+)N-allyl-N-normetazocine; MK 801, (+)-5-methyl-10,11-dihydro-5H-dibenzo[a,d]cyclohepten-5,10-imine maleate; and TCP, 1-[1-(2-thienyl)cyclohexyl]piperidine, on the norepinephrine uptake] system. The inhibition of norepinephrine uptake by these agents was revealed as a potentiation of the contractile response to norepinephrine or transmural nerve stimulation, an effect which was blocked by cocaine, but not deoxycorticosterone (DOC). In the presence of norepinephrine uptake blockers (cocaine and DOC), a third action of (+)3PPP and (+) SKF 10047 was unveiled, an inhibition of the contractile response to norepinephrine, possibly via sigma receptors. The order of potency of additional sigma ligands, which also inhibit contractile responses to norepinephrine; haloperidol; DTG, 1,3-di-ortho-tolyl-guanidine; BW 234U, cis-9-[3-(3,5-dimethyl-1-piperazinyl)propyl]carbazole dihydrochloride (rimcazole); and BMY 14802, a-(4-fluorophenyl)-4-(5-fluoro-2-pyrimidinyl)-1-piperazine butanol; suggests that sigma receptors may be present in blood vessels and could play a role in modulating contractile responses.

Supported by NIH grant #DK36289 and Fellowship #DA05359-02

485.20

DEXTROMETHORPHAN DISTINGUISHES BETWEEN [3H] DTG AND [3H] (+)-3-PPP LABELED SIGMA RECEPTOR BINDING SITES IN GUINEA PIG BRAIN MEMBRANES. K. Naper*, M. Pontecorvo, W. Karbon*. (Sponsor: S.J. Enna) Nova Pharmaceutical Corp., Baltimore, MD.

The ability of compounds to interact with sites labeled by sigma receptor ligands was examined in whole guinea pig brain membranes. Haloperidol exhibited equal affinity (Ki = 4-6 nM) for sites labeled by [3H] DTG and [3H] (+)-3-PPP, while DTG was 2-fold more potent against [3H] DTG (Ki = 30 nM), and (+)-3-PPP was 3-fold more potent against [3H] (+)-3-PPP (Ki = 36 nM). In contrast, dextromethorphan (DM) was 20-fold more potent against [3H] (+)-3-PPP (Ki = 0.12 μM) than [3H] DTG (Ki = 2.6 μM). Likewise, caramiphen displaced [3H] (+)-3-PPP with a potency (Ki = 9 nM) 10-fold greater than observed for [3H] DTG (Ki = 92 nM). DM also interacted with sigma receptor binding sites labeled with [3H] haloperidol and [3H] SKF-10,047, exhibiting a potency vs [3H] haloperidol (Ki = 3.8 μM) similar to that observed vs [3H] DTG, and a Ki = 0.18 μM against [3H] SKF-10,047, similar to that observed vs [3H] (+)-3-PPP.

Phenytoin (300 μM), which is known to enhance [3H] (+)-3-PPP binding, also enhanced [3H] SKF-10,047 binding but did not increase the binding of [3H] DTG or [3H] haloperidol. In addition, phenytoin enhanced the potency of DM to displace both [3H] DTG and [3H] (+)-3-PPP.

These findings suggest that sigma receptor ligands bind to either distinct sites, or different confirmations of the same site which are distinguished by DM but not by haloperidol.

485.21

5-HT FUNCTION IN THE BIOCHEMICAL AND BEHAVIORAL RESPONSES TO MCPP IN HEALTHY SUBJECTS AND SCHIZOPHRENICS. J.P. Seibyl*, J.H. Krystal, L.H. Price, S.W. Woods, G.R. Heninger, D.S. Charney. Yale University School of Medicine, West Haven VAMC, West Haven, CT 06516.

Research with 5-HT2 agonists have been limited by hallucinogenic properties. M-chlorophenylpiperazine (MCPP) acts at both 5-HTl and 5-HT2 receptors in animals. We evaluated the ritanserin (5HT2 antagonist)-reversible component of MCPP effects in healthy human subjects and responses to MCPP alone in neuroleptic-free schizophrenics (SCHIZ). METHODS: In an ongoing study, subjects completed 4 test days in a randomized order: 1) Placebo (PLA)-MCPP (0.1 mg/kg iv), 2) Ritanserin (5 mg po; or 10 mg po)-MCPP, 3) PLA-PLA, 4) Ritanserin-PLA. SCHIZ's (n=5) completed MCPP and PLA test days. RESULTS: Ritanserin attenuated MCPP-induced increases in prolactin, growth hormone, cortisol, drowsiness, feeling high, and anxiety. SCHIZ's experienced significant MCPP-induced increases in Brief Psychiatric Rating Scale psychotic symptoms; negative symptoms were unchanged. Two SCHIZ's (1 with, 1 without previous vis. hall.) experienced visual hallucinations after MCPP, but not PLA. Anxiety increases preceded worsening of positive symptoms. COMMENT: MCPP has 5-HT2 agonist effects in healthy subjects and SCHIZ's and may be a clinically useful 5-HT2 probe.

485.22

IN VIVO CONCENTRATIONS OF INOSITOL PHOSPHATES IN RAT BRAIN REGIONS: EFFECTS OF ISCHEMIA, LITHIUM AND SEIZURES. R.S. Jope, R.E. Smith*, R.A. MacQuarrie*, University of Alabama, Birmingham, AL 35294 and University of Missouri, Kansas City, MO 64108.

Second messengers derived from phosphoinositide hydrolysis are important components of signalling mechanisms in many cell types. A new anion exchange chromatographic method was applied in combination with focussed beam microwave irradiation to measure the in vivo concentrations of endogenous, unlabelled inositol mono-, bis-, tris- and tetrakisphosphate in rat brain cerebral cortex, hippocampus and striatum.

The distribution of the four inositol phosphates was similar in each of these three brain regions, with IP_1 predominating. Ischemia produced by decapitation caused widespread changes, most notably a loss of inositol trisphosphate. Chronic lithium treatment caused large reductions in the concentration of IP_3. Acute lithium treatment generally only caused small changes in the inositol phosphates, and these varied among the regions.

Administration of pilocarpine to lithium-treated rats induced seizures and rapid, large increases of IP_3 and IP_4. Supported by MH38752.

485.23

Evaluation of a Receptor Binding Assay for Determining Anticholinergic Drug Levels in Plasma. W. D. Horst, C. Stucky*, P. Widener*, S. Castellani* and S. H. Preskorn, VA Med. Ctr, Univ. of KS Sch. of Med.-Wichita, and Psychiatric Research Institute at St. Francis Reg. Med. Ctr, Wichita Kansas

3H-QNB radioreceptor binding assays can be used to determine plasma anticholinergic drug levels (Tune & Coyle, Psychopharmacol 75, p. 9, 1981); however, technical difficulties prevent routine use: binding of the radioligand to plasma proteins lower effective ligand concentration leading to overestimation of activity; plasma protein binding of anticholinergic drugs may result in an underestimation. We evaluated these factors in a 3H-QNB binding assay (Yamamura and Snyder, Proc. Nat. Acad. Sci. USA, 71, p.1725, 1974). Approximately 20% of the 3H-QNB was bound to plasma protein and was constant over incubation periods of 0.5 to 3 hours and unchanged in the presence of amitriptyline (800 ng/ml). Amitriptyline concentration curves in buffer and in plasma were similar, suggesting 3H-QNB binding reflects total plasma anticholinergic activity. The IC20's for amitriptyline, thioridazine and desipramine are 50, 120, and 350 ng/ml plasma respectively, providing sufficient sensitivity at average therapeutic plasma concentrations. Based on these observations, this assay could be clinically useful to monitor plasma anticholinergic levels in patients being treated with anticholinergic drugs. This study was supported by VA RAGS Grant #103.

485.24

BIOLOGICAL ACTIVITY OF FENTANYL ANALOGS. David W. Smith*, Duncan P. Taylor, Judith M. Libera*, and Stacy N. Suberg. CNS Biology, Bristol-Myers Company, P.O. Box 5100, Wallingford, CT 06492-7660.

Fentanyl is a potent synthetic opiate analgesic with rapid onset and short duration. A series of compounds were synthesized as analogs of fentanyl. These compounds may be considered as N-($aryl_1$)-N-[1-(2-$aryl_2$ethyl)-4-piperidinyl] propanamides, with the substitutions shown in the table.

Compound	$Aryl_1$	$Aryl_2$
Fentanyl	Phenyl	Phenyl
A	Thieno[2,3-d]pyrimidin-4-yl	Phenyl
B	1,2-Benzisothiazol-3-yl	Phenyl
C	1,2-Benzisothiazol-3-yl	2-Thienyl

We have explored their biological activity in both receptor binding and behavioral assay systems. In vitro and in vivo profiles revealed that all were potent displacers of μ-opiate binding, with a broad spectrum of "sodium shift" ratios; several were potent κ-opiate displacers, and all were potent in the mouse hot plate (i.p.) and mouse phenylquinone writhing (PQW) (s.c.) tests.

Compound	μ(-Na)	μ(+Na)	κ	PQW	Hot Plate
Fentanyl	4	30	480	--	0.5
A	21	49	2200	0.29	6.0
B	16	40	73	0.66	7.8
C	15	56	44	0.26	8.0
Morphine	2	19	49	0.62	7.5

485.25

QUANTITATIVE GRIP STRENGTH ASSESSMENT AS A MEANS OF EVALUATING MUSCLE RELAXATION IN MICE. M.E. Nevins, S.M. Arnolde* and P.M. Beardsley*. CNS Diseases Research, G.D. Searle & Co., Skokie, IL 60077.

The quantitative assessment of grip strength (GS) in rodents is used by neurotoxicologists as one means of evaluating potential neuromotor effects of enviornmental and pharmacological agents. Various forms of GS assessment have also been used in the specific evaluation of the muscle relaxant properties of drugs. The current study explored the effects of several muscle relaxants (s.c.) on the forelimbGS of CD-1 mice using a procedure described by Meyer et al. (Neurobehav. Toxicol. 1:233-6, 1979). This procedure utilizes a strain gauge to measure the lateral pull of force, in grams, exerted by mice as an index of muscle relaxation. Other drugs such as anesthetics, major tranquilizers, stimulants, etc. were also tested in order to determine the specificity of the effects of the muscle relaxants in this paradigm. The muscle relaxants diazepam, midazolam, baclofen, methocarbamol and dantrolene dose-dependently reduced forelimb GS. 2-Amino-7-phosphonoheptanoic acid, which has been shown to have muscle relaxant effects, also reduced GS. Pentobarbital, ethanol (i.g.), phencyclidine, ketamine and chlorpromazine also reduced GS but at behaviorally-impairing doses. Lithium chloride, at doses typically used to induce taste aversions, and clonidine, at doses that reduce locomotor activity, did not affect GS. In addition, stimulant doses of amphetamine and caffeine, but not of morphine, increased GS in a dose-dependent manner. These results extend the findings of Meyer et al., and suggest that this forelimb GS procedure may be useful for the evaluation of the muscle relaxant properties of drugs.

CLINICAL CNS NEUROPHYSIOLOGY

486.1

INHOMOGENEITY AND BOUNDARIES IN ELECTRICAL CONDUCTIVITY CAN SIGNIFICANTLY MODIFY THE MAGNETIC EVOKED FIELD DUE TO NEURONAL CURRENTS. Y.C. Okada, J.-C. Huang* and C. Nicholson. Dept. of Physiology and Biophysics, New York Univ. Med. Center, NY 10016.

The magnetic evoked field (MEF) is due to currents directly associated with active neurons plus those due to inhomogeneity in electrical conductivity of the tissue. We studied the conditions under which tissue inhomogeneity may significantly modify the MEF due to neuronal currents, utilizing the isolated cerebellar preparation of turtle (Okada et al., Brain Res. 421:151, 1987). The extracellular conductivity (σ_e) of this lissencephalic, oblate spheroidal structure was determined from the Ohm's law by applying a current of known density perpendicular to cerebellar surface and measuring the induced electric field in depth. The σ_e was a complex function of depth: σ_e = 0.15 (sd=0.03) and 0.25 (sd=0.04) Siemens/m at 21°C in the granular and molecular layers, respectively. The σ of the Ringer was 1.33 S/m (sd=0.18). A boundary element method indicated that the MEF due to neuronal currents to be enhanced by a factor of 2.3 by the cerebellar surface boundary and reduced by a factor of 1.5 by the conductivity change from the molecular to the granular layers. These large effects arise from the proximity of neuronal sources to the boundaries (<0.5 mm). The conductivity boundaries primarily affect magnitude of the MEF, inasmuch as neuronal source locations are stationary in time, but it could also modify the temporal waveform if the source location moves in depth as in the case of cortical sources. Supported by NINDS grant NS21149.

486.2

TRANSMEMBRANE POTENTIAL, EXTRACELLULAR FIELD POTENTIAL AND MAGNETIC EVOKED FIELD IN RESPONSE TO APPLIED ELECTRIC FIELD IN THE ISOLATED TURTLE CEREBELLUM. L. Lopez* (SPON: M.A. Nathan), C.Y. Chan•, Y.C. Okada and C. Nicholson. Dept. of Physiology and Biophysics, New York Univ. Med. Center, NY 10016 •Dept. of Physiology, CUNY Med. School, NY 10031.

The differential polarization of dendritic and somatic regions of the Purkinje cells (Pc) in the isolated turtle cerebellum (Chan C.Y. and Nicholson C., J. Physiol., 371:89, 1986) was achieved by a uniformly applied electric field across the preparation immersed in Ringer. We measured, in two experimental sets, the extracellular field potential (EP) together with the magnetic evoked field (MEF) (Okada Y.C. et al., Brain Res., 421:151, 1987); and the EP together with the transmembrane potential (TMP) (Chan C.Y. et al., J.Physiol., 402:751, 1988). Two stimuli were used: pulsed field (.2-.3 ms) and sinusoidal field (2, 5, 10 Hz). Pulse. The EP consisted of two negative components (latencies: 1.4 and 3 ms). The threshold was lower for dorso-ventral stimulus (dv). The intracellular recording of Pc dendrite showed climbing fiber (cf) response at 3.4 ms; at the soma the direct activation of the Pc was seen at 1.4 ms. These latencies are consistent with direct activation (1.4 ms) and monosynaptic transmission (3.4 ms) in turtle. The corresponding MEF was consistent with currents flowing from soma to dendrite of the Pc. Sinusoid. Both EP and MEF showed two components at dv similar to the ones evoked by the pulse. The ventro-dorsal stimulus (vd) showed a dendritic-spike response. The TMP confirmed the nature of these components as Na spikes and cf-evoked response respectively for dv and to Ca spikes for vd. In both experimental sets, Kynurenic acid blocked the post-synaptic component and not the directly activated Na currents that were Tetrodotoxin-sensitive. The use of the sinusoidal field clearly pointed out the asymmetric activation by the two stimulation polarities due to differential polarization of dendritic and somatic regions. More important, we show that applied fields can elicit synaptic responses through cf activation. The pulse-evoked cf-responses resemble those evoked by peduncular stimulation. Furthermore, the correlation with TMP demonstrates the ionic origin of the recorded MEF.

Supported by NINDS grant NS21149.

486.3

EFFECTS OF CHOLINERGIC DRUGS ON THE MIDLATENCY AUDITORY POTENTIAL "WAVE A" IN CATS. J.B. Harrison and J.S. Buchwald. Brain Res. Inst., Ment. Ret. Res. Ctr., Dpt. of Physiol., UCLA Med. Ctr., Los Angeles, CA 90024.

The midlatency (20-30 msec) positive auditory evoked potential wave A in the cat appears to be a correlate of P1 in humans. Both potentials are absent at rapid click repetition rates (10/sec) and during slow-wave sleep but present during wakefulness and REM sleep. We previously showed that wave A is eliminated by bilateral lesions of the pedunculopontine tegmental nucleus in the midbrain reticular formation. This nucleus contains cholinergic cells, and we have also shown that scopolamine, a muscarinic cholinergic antagonist, eliminates wave A. To test further the hypothesis that wave A is dependent on cholinergic cells, we studied the effects of cholinergic agonists. EEG was recorded, with amplifier filter settings at 10 Hz- 3 kHz, from awake cats, with clicks presented at a rate of 0.2/s. In all cats tested, scopolamine (0.3 mg/kg, subcutaneous) eliminated wave A. Both carbachol (.03 mg/kg, subcutaneous), a muscarinic agonist, and physostigmine (.25 mg//kg, intravenous), a cholinesterase inhibitor, reversed this scopolamine effect, i.e., the eliminated wave A returned. Control intravenous injections of the same volume (2 cc) of carrier did not have these effects. Nicotine bitartrate (0.1 mg/kg, intravenous) had no effect on wave A. These results extend prior work, all of which supports the hypothesis that wave A is dependent on muscarinic cholinergic activity. (Supported by USPHS Grants HD05958 and NS25400).

486.4

DIFFERENTIAL ABNORMALITY OF "P1" MIDLATENCY AUDITORY EVOKED RESPONSE IN ALZHEIMER'S DISEASE, J. Buchwald, R. Erwin, S. Read, D. Van Lancker and J. Cummings, Depts. of Physiol, Psychiatr., Neurol., Brain Res. Inst., Ment. Retard. Res. Center., UCLA Medical Center, Los Angeles, CA

The human MLRs consist of a 30-40 msec positivity, "Pa", a 40-50 msec negativity, and a subsequent 50-65 msec positivity, "P1". The human P1 and the cat MLR, "wave A", both disappear as click rates exceed 1/sec and during slow-wave sleep; both increase during REM sleep to equal their amplitudes during wakefulness. Additional data in the cat support the hypothesis that the human P1 and cat wave A are generated by cholinergic cells within the ascending reticular arousal system (RAS) projecting to cholinoceptive target cells in the thalamus. Insofar as forebrain cholinergic dysfunction occurs in Alzheimer's disease (AD), we postulated that brainstem cholinergic dysfunction might likewise occur and be reflected by an abnormal P1. In order to test this prediction, the following study was carried out on 6 AD men, mean age 63 years. Probable AD (N=2) was diagnosed using NINCDS-ADRDA guidelines. Definite AD (N=4) was diagnosed following cortical biopsy which demonstrated confirmatory plaques and neurofibrillary tangles. An age-matched group of healthy, neuropsychiatrically normal men served as controls. Click stimuli were presented binaurally at an intensity 55 db above hearing threshold for each subject. MLRs were recorded from a midline scalp electrode referenced to linked mastoids with filter bandpass of 10-300 Hz. Comparisons between AD and age-matched control groups indicated normal auditory brainstem and Pa responses. In contrast, P1 was missing or dramatically reduced in the AD subjects. While the present sample size is small, this first demonstration of significant P1 reduction in AD suggests RAS dysfunction involving cholinergic cells of the brainstem.

486.5

A LEFT HEMISPHERIC DEFICIT IN P200 FROM ALZHEIMER'S PATIENTS RECORDED IN A VISUAL MEMORY TASK. S. Sands, J. De La Chapa, and S. Smith. Dept. of Psychology, Univ. Texas at El Paso, El Paso, Tx, 79968.

A voltage deficit maximal in the left hemisphere was observed in Alzheimer's Disease (AD) patients performing a serial probe recognition (SPR) task compared to age match controls. Six suspected Alzheimer's patients meeting NINCDS-ADRDA selection criteria were selected from a larger population of dementia patients. They were compared to15 control subjects free of any history of neurological or cognitive impairing disorders. Event-related potentials (ERPs) were recorded from 28 electrode sites (modified international10-20 system) while subjectsperformed a variable list length (1,4 and 8 item) SRP task. Ocular artifact was monitored via VEOG and HEOG leads. ERPs were averaged separately for list and probe items. A reduction in P200 amplitude was observed in left-hemispheric recording sites (i.e., F3,C3,T3,T5) from the AD patients. This effect was not observed in controls subjects who did poorer in the memory task. These results are consistent with recent PET findings of increased left-hemispheric abnormalities in suspected AD

486.6

VISUAL ELECTROPHYSIOLOGICAL AND MORPHOLOGIC CORRELATES IN THREE VARIANTS OF HUMAN NEURONAL CEROID LIPOFUSCINOSIS, Arnold A. Lidsky, K.E. Wisniewski*, O.F. Patxot*, Clinical Electrophysiology of Vision Lab., NYS Institute for Basic Research, Staten Island, N.Y. 10314

Neuronal ceroid lipofuscinosis (NCL) is a neurodegenerative disease, typically presenting with progressive cerebral and visual dysfunctions. Clients were tested for electroretinograms (ERG) and visual evoked responses (VEP) to light flashes, using computer averaging of corneal ERGs and occipital scalp VEPs. Skin punch biopsies were taken for diagnostic confirmation. Five Infantile cases, three Late Infantile, and six Juveniles had ERGs and VEPs performed at ages 26 to 290 months. Juvenile ERGs were most abnormal, with extremely delayed a- wave peaks and smallest amplitude b- waves, despite near normal VEPs; biopsies indicated fingerprint-like granular inclusions. In contrast the Late Infantiles (age 76 months) showed nearly normal a- and b-patterns with slowest P100 peak latencies (146-216 ms) and largest VEP amplitudes; curvilinear inclusions were typical. Finally the Infantile cases (age 77 months) showed delayed N75 peaks in VEPs with significantly reduced amplitudes both in scotopic b waves of the ERG and in the VEP; granular inclusions were common. The visual electrophysiological data thus suggest peripheral retinal disorders characterized the Juveniles, while CNS was most affected in Late Infantile cases. These relationships are being extended in serial retesting of ERGs and VEPs, and in our expanding data base.

486.7

CHARACTERIZATION OF MOTOR EVOKED POTENTIAL IN THE SPINAL CORD INJURED RAT. Y.G. Park* (Sponsored by B. Green), J.H. Kim, R. Prado, V.R. Holets, J.S. Cheon, W.J. Levy. Dept. of Neurological Surgery, Univ. of Miami, Sch. of Med., Miami, FL 33136.

The motor evoked potential (MEP) is a new neurological test utilizing motor cortex stimulation to evoke potentials which can be monitored along the spinal cord. In clinical use, the MEP has been more reliable than the somatosensory evoked potentials for predicting recovery of motor function after spinal cord injury. The goal of this study was to develop a technique for monitoring MEPs chronically in the spinal cord of rats, and to test if the MEP is correlated with functional recovery of hindlimb walking after spinal cord injury.

Two pairs of teflon coated wire electrodes with an exposed tip of 1 mm were chronically implanted on the dural surface at T6 and L1. Wires were guided subcutaneously and connected to a multipin plug fixed on top of the cranium. Electrical stimulation of SM cortex produced MEP which consisted of 2-4 consecutive positive peaks with 0.3 msec interval. The earliest D wave generally required higher intensity of stimulation than the second or third waves. The conduction velocity of the D wave was 70-90 m/sec. The amplitude of the D wave monitored at different sessions varied slightly, but the ratio of the amplitude of the D waves at T6 and L1 (L/T) level remained constant in the same animal over the 10 week period. The L/T ratio of D wave declined to the size of the lesion produced between the two recording sites (T8), improved for 3 weeks post lesion. The improvement of the L/T ratio was correlated with the recovery of hindlimb locomotion.

486.8

CHARACTERIZATION OF CEREBELLAR EVOKED POTENTIAL IN RAT. J.S.Cheon, J.H. Kim and W.J.Levy. Dept. of Neurological Surgery, Univ. of Miami, Sch. of Med., Miami, FL 33136.

The cerebellar evoked potential (CEP) has been proposed as a clinical tool to assess the integrity of the extrapyramidal system. Electrical stimulation of the cerebellar cortex produces 2-3 positive waves traveling in the ventral cord. However,the pathways producing these potentials remain to be investigated. The purpose of this study was to characterize the components of the CEP in the rat and to clarify the pathways. The rat was placed in a stereotaxic head holder and craniotomy was performed to expose cerebellar cortex. Two laminectomies were performed to expose the T6 and L1 segments of the spinal cord. Epidural recordings were made with a pair of teflon coated stainless steel wire electrodes with 1 mm exposed tips. The cerebellum was stimulated by two screws placed bilaterally on the occipital bone on top of the intermediate zone in lobule 6. The CEPs recorded on the thoracic cord consisted of several positive peaks with conduction velocities of 30-100 m/sec, but the potentials recorded on the lumbar cord showed only two positive peaks with conduction velocities of 23m/sec and 45m/sec. Dorsal hemisection of the spinal cord between the two recording electrodes did not change the components of the CEP monitored at T6 or L1. However, the amplitude of the CEP monitored at the lumbar cord declined. Effects of selective lesion of red nuclei, and vestibular nuclei, are under investigation.

486.9

COVARIANCE AMONG MOTOR EVENTS IN SCHIZOPHRENIA: SMOOTH PURSUIT EYE MOVEMENTS, SPINAL REFLEXES AND REACTION TIME. R.T. Pivik, F.W. Bylsma* and P.M. Cooper*. Dept. of Psychiatry, Univ. of Ottawa and Ottawa General Hospital, Ottawa, Ont. K1H 8L6.

The present study examined the extent to which motor behaviors, shown to be deviant in separate populations of schizophrenics, would covary in the same patients. Motor activities studied included smooth pursuit eye tracking, variations in excitability of the spinal monosynaptic H-reflex, and button-press reaction time (RT) to an auditory tone. These measures were assessed in: actively-ill schizophrenics (n=17); schizophrenics in remission (n=12); and, normal controls (n=17). Diagnosis and clinical status were determined independently by two psychiatrists using DSM-III criteria, interviews and hospital records. Measures were recorded using standardized techniques, electronically processed and evaluated using analysis of variance procedures. For all measures, actively-ill patients deviated from comparison groups, demonstrating enhanced reflex excitability, impaired tracking and slow RTs. With the exception of the RT comparison with remitted patients, these differences were statistically significant. The results indicate deviant responses at several levels of motor functioning in actively-ill schizophrenics. Furthermore, since the degree of abnormality abates considerably in remitted schizophrenics, to a great extent these effects appear to be state dependent.

Research assisted by the Ontario Mental Health Foundation.

486.10

THE EFFECT OF LOW DOSE INTRATHECAL MORPHINE ON SPINAL SPASTICITY IN HUMAN SUBJECTS WITH SPINAL CORD LESIONS. R.M. Herman, Samaritan Rehabilitation Institute, Phoenix, AZ 85006.

Recently, we have reported that low dose intrathecal (i.t.) morphine (MOR) causes naloxone reversible enhancement of bladder capacity due to inhibition of vesico-vesical reflexes and potentiation of vesico-somatic (sphincter) reflexes in patients with suprasacral spinal cord lesions (SSCL). Our present studies examine the effect of randomly delivered i.t. MOR (100, 200, 400 μg) on spasticity among three (3) complete and five (5) incomplete SSCL patients. Spinal spasticity was characterized by multisegmental or irradiating motor (EMG) contractions of bilateral proximal and distal lower limb muscles, occurring spontaneously or induced by vesical and cutaneous (nociceptive and non-nociceptive) stimulation. All doses of i.t. MOR caused a) profound suppression of motor reactions which appeared considerably later than the alteration of vesical reflexes, and b) suppression of spontaneous and vesical induced contractions for 40-60 hours, while duration of inhibition on reflexes evoked by cutaneous stimulation endured for 20-26 hours. Naloxone produced marked reversal of cutaneous reflexes with little or no effect on spontaneous or vesical induced limb motor responses. These observations are ascribed to release of MOR sensitive opioid (and non-opioid) μ receptor sites by spinal lesions.

486.11

DIABETIC ENCEPHALOPATHY: CAN IT BE PREDICTED FROM THE RETINAL STATUS? M.A. Kabene*[1], J. Everett*[2], C. Harnois*[3] (SPON: Harold W. Gordon). School of Psychology[1,2], and Department of Ophthalmology[3], Laval University, Quebec City, Canada.

Different complications are related to diabetes, and among them is retinopathy. Few things are known about the possible effects of diabetes on the brain. The physiological and vascular similarities between the brain and retina suggest that the brain could be affected in the same way by diabetes. Neuropsychological measures such as the Hooper Visual Organization test, the Category test, the Wechsler Memory Scale, the Stroop test and the Trail Making test A and B were used to assess the brain integrity. Three groups of subjects were included in our study: 52 controls, 44 diabetics without retinopathy and 34 diabetics with retinopathy. The diabetic with retinopathy consisted of 21 diabetics with an early diabetic retinopathy and 10 with preproliferant or proliferant retinopathy. Our results suggest that neuropsychological deficits can exist even before the retinopathy, but that they are worse after the onset of retinopathy. The severity of retinopathy seems similar in magnitude to encephalopathy as determined by the neuropsychological deficits.

486.12

ENHANCEMENT OF CORTICAL EVOKED POTENTIALS BY ETOMIDATE: LOCUS AND POSSIBLE MECHANISM. S.K. Samra and L.S. Sorkin, Depts. of Anesthesiology, Anat. and Neurosci., Univ. of Texas Med. Branch at Galveston, Galveston, TX 77550.

In contrast with all clinically used anesthetics, which are known to decrease the amplitude (ampl) of cortical evoked potentials (CEP), etomidate has been shown to enhance CEP in humans. This study attempts to define the locus and possible mechanism of this phenomenon.

Cats were anesthetized with halothane (0.5-1%) in a mixture of 50% N_2O in oxygen. Blood pressure (BP) was continuously monitored. Core temperature and end-tidal CO_2 were maintained within physiological limits. Evoked potentials were recorded from VPL thalamus and either SI or SII sensory cortex following tibial nerve stimulation.

Etomidate (1-3 mg/kg) caused a transient, dose dependent decrease in BP which was accompanied by a decrease in ampl of CEP (both N_1 and P_1). Return to normal BP was accompanied by 50-150% increase in ampl. of CEP compared to control tracings. Pretreatment with GABA antagonists and agonists modified the etomidate enhancement of CEP. Thalamic recordings remained unchanged throughout. These results suggest that enhancement of CEP is occurring within the cerebral cortex and involves GABA receptors. (Supported by NIH grant N.S. 11255).

486.13

AUDITORY LONG LATENCY ERPs IN RATS: REGIONAL AND NEUROCHEMICAL FINDINGS; C.L. Ehlers, T. L. Wall , R.I. Chaplin*. Department of Neuropharm., R.I.S.C., La Jolla, CA 92037

Animal models of event related potentials (ERPs) have recently been developed in order to gain further understanding of the physiological variables which underlie these brain potentials. The present study utilized unanethetized rats in order to: 1) evaluate whether a P3-like component could be identified in this animal in response to variations in the stimulus characteristics of an auditory oddball paradigm; 2) compare ERPs from different brain sites; 3) test the effects of amine depletion on ERP morphology. Sixty-one male Wistar rats were used in the study. The results of these studies showed that all electrode sites tested (cortex, nucleus acumbens, amgydala, dorsal hippocampus, locus coeruleus) contain large amplitude potentials in the 50-100 msec latency range which were sensitive to changes in stimulus characteristics such as probability and loudness. Whereas late positivities in the 300-400 msec latency range were only identified in the dorsal hippocampus and in the amygdala. Destruction of the dopamine containing neurons of the VTA using 6-OHDA was not found to produce any changes in ERP responses of any of the components in the lesioned rats as compared to the sham animals. Whereas the serotonin depletion produced by PCPA injections was found to produce significant reductions in the amplitude of the N1-like component in cortex. Dorsal noradrenegic bundle lesions produced by 6-OHDA also caused significant changes in ERP components. Lesioned animals were found to have increases in amplitude of the N1-like potentials in response to frequent tones in cortical leads and decreases in the amplitude of the "P3-like" potential in hippocampal leads in response to infrequent tones. This finding is consistent with a role for NE in the hippocampus in the processing of novel or selective stimuli. These studies suggest that the rat may be a good model for further exploration of long-latency ERPs. (Supported by NIAAA 00098, 06059)

486.14

SOMATOSENSORY CORTICAL POTENTIALS EVOKED BY MAGNETIC STIMULATION OF THE MEDIAN NERVE IN MAN. S.S. Haghighi and J. Ebeling*. Division of Neurosurgery, University of Missouri-Columbia, Columbia, MO 65202.

Cortical somatosensory evoked potentials (SSEPs) were studied by the percutaneous magnetic coil (PMC) or standard electrical stimulation of the distal median nerve in normal subjects and patients with peripheral neuropathy.

Cortical peak latencies of N1 and P2 were recorded in all normal subjects. The mean latency of N1 and P2 peaks were 20± 2.23 and 22.5± 2.17 msec respectively for the conventional electrical stimulation. These values were 20.2± 1.92 and 22.3± 2.16 msec for N1 and P2 peaks when the PMC stimulation was used. No significant difference in the peak latency, amplitude, or configuration of the median nerve SSEPs was noticed between the magnetic and the electrical stimulation. The patients with peripheral neuropathy showed a delayed peak latency and slowing of conduction velocity. We concluded that the PMC stimulation can elicit cortical evoked responses and it can detect lesions of peripheral nerves or spinal cord. The disadvantages of PMC stimulation were lack of precision in defining exact site of the nerve activation, and prolonged averaging time due to the slow capacitor discharge of the magnetic stimulator.

486.15

CHANGES IN HUMAN CORTICAL MOTOR REPRESENTATION AREAS OF MUSCLES PROXIMAL TO THE STUMP AFTER AMPUTATIONS: A STUDY WITH TRANSCRANIAL MAGNETIC STIMULATION . L. G. Cohen and M. Hallett. Human Motor Control Section, Medical Neurology Branch, NINDS, NIH, Bethesda, MD 20892.

To evaluate reorganization of body part representations in human motor cortex after amputations, we studied motor evoked potentials (MEP) to transcranial magnetic stimulation (TCMS) in seven patients with unilateral upper limb amputations and in 10 controls. Electromyographic recordings were made from muscles immediately proximal to the stump and the same contralateral muscles. TCMS was delivered by a Cadwell MES-10 magnetic stimulator through a "butterfly-shaped" coil over scalp positions separated by 1.0 to 2.5 cm. Supramaximal muscle responses were elicited by peripheral nerve stimulation. Amplitude of MEP to TCMS was expressed both as absolute values and as a percentage of maximal responses to peripheral nerve stimulation. Threshold for excitation of muscles ipsilateral and contralateral to the stump and the region of excitable scalp positions were also determined in five patients.

TCMS evoked larger MEPs ($p \leq 0.01$), recruited a larger percentage of the motoneuron pool ($p \leq 0.008$), and evoked MEPs at lower intensities of scalp stimulation ($p \leq 0.006$) for muscles ipsilateral to the stump than for those contralateral. Muscles ipsilateral to the stump could be activated from a larger area than those contralateral to the stump ($p \leq 0.01$). These results are compatible with more or stronger corticomotoneuronal connections spread over larger cortical areas targeting muscles proximal to the stump than those on the normal side.

486.16

MULTI-MODAL STEREOTACTIC IMAGING FOR MAPPING BRAIN FUNCTIONAL IMAGING TO ANATOMIC STRUCTURE. J.X. Zhang, C. Wilson, M. Levesque, R.M. Harper and J. Engel. Brain Res. Inst. and Depts. of Neurology, Neurosurgery, and Anatomy & Cell Biology, UCLA, Los Angeles, CA 90024.

Positron emission tomography (PET) provides an index of functional activity of brain structures. However, it is difficult to coordinate PET functional maps with anatomical boundaries because of the poor spatial resolution of PET images and the poor anatomical differentiation from radioactive emission PET. We developed a multi-modal imaging system based on a modified Leksell stereotactic frame and a MicroVAX/GPX computer that allows for simultaneous display from PET, magnetic resonance imaging (MRI), computerized tomography (CT), and digital subtraction angiography (DSA) images. Based on the external fiducial markers attached to the frame, different images are adjusted to a common 3-dimensional coordinate system. The system allows regions of interest defined in one image to be transferred to another image modality. A brain structure outlined in MRI or CT images can easily be mapped to a PET image. Conversely, a functional region contoured in a PET image can be categorized into anatomic structure in MRI and CT images. The procedure has been applied to localize tissue function and structure in focal epilepsy of temporal lobe origin.

486.17

MEG (MAGNETOENCEPHALOGRAPHY) MAPPING OF AUDITORY EVOKED STEADY-STATE RESPONSES IN ALZHEIMER PATIENTS.
U. Ribary, R. Llinás, A. Kluger*+, J. Suk* and S.H. Ferris+ Dept. of Physiology & Dept. of Psychiatry, New York University Medical Center, New York, NY 10016 U.S.A.

A 14-multichannel neuromagnetic measuring system was used to analyze the dynamics and source localizations of the steady-state magnetic components of the electrical response evoked during a 40 Hz continuous auditory stimulation in controls and Alzheimer patients (60-75 years). Mapping of magnetic (MEG) fields was done over the right hemisphere utilizing an average of 500 epochs of 100 msec in duration. This steady-state response to monotonal auditory stimulation was analyzed at 1 msec steps, such that the dynamic component of the response may be determined. This sinusoidal auditory MEG data in control subjects indicate a stereotyped temporal pattern of magnetic activity over the hemisphere. It consisted of a positive-negative field sequence which rotated from dorso-posterior to ventro-anterior cortical areas in a continous phase shift manner over parietal, temporal and frontal areas. The largest amplitudes for this activity was found over temporal cortex. Source localization, using a single-dipole model, indicates that this magnetic wave reflects a complex, time locked sequence of network activations involving most probably thalamo-cortico-thalamic pathways. In Alzheimer patients, decreased magnetic fields over time and a reduction of the rotational speed of their response was observed. This was largely due to increased phase shifts over the hemisphere and to a lower activity in fronto-pariotemporal areas. In addition, source localization indicates an overall reduction in cortical activity and of the synchronization of 40 Hz activity over the sensory area in these patients.

These data illustrate a new approach to monitoring the dynamics of pathological brain function in psychiatry. Indeed, the 40 Hz response may develop into a powerful tool as an early and more sensitive marker for Alzheimer's disease.

486.18

P3 LATENCY REFLECTS COGNITIVE STATE IN EARLY HEPATIC ENCEPHALOPATHY. N.E. Noldy*, P.A. Cleland*, B. El-Nesr*, R.D.G. Blair, & P.L. Carlen. Addiction Research Foundation, Toronto, Ontario M5S 2S1 CANADA.

Early detection of encephalopathy in patients with liver disease may prevent further loss of brain functioning. This study examines the possibility that the latency of P3, a cognitive event-related potential, might aid in the diagnosis of early hepatic encephalopathy (HE). Since P3 latency is associated with speed of information processing, it might be sensitive to the subtle cognitive decline which characterizes the early stages of HE.

Patients presented with alcoholic liver disease and Grade 1 or 2 HE. P3 latency, as recorded in a standard auditory oddball paradigm, was compared with two measures which have proven sensitive to early HE in some patients: dominant EEG frequency and Reitan's Trail Making (B) task.

The dominant quantified EEG (qEEG) frequencies in this population ranged from normal alpha to theta range. Similarly, Trail Making scores ranged from perfectly normal to impaired and the latency of P3 also ranged from normal to +3 SD above the mean. P3 latency was inversely related to dominant qEEG frequency and was strongly correlated with the cognitive Trail Making task. Thus, P3 latency may prove an important measure to include among indices of preclinical HE, particularly in patients with motor deficits which interfere with psychological tests such as trail making.

486.19

INDICATIONS OF A TEMPORAL LOBE ORIGIN OF THE P300 WAVE. S.B. Seminara*, and S. Khoshbin. EEG Lab., Brigham and Women's Hospital, Harvard Med. Sch., Boston, MA 02115.

Studying the topography of the P300 attention wave, cortical auditory evoked potentials (AEPs) to frequent (90%) 1,000-Hz and infrequent (10%) 2,000-Hz 50 msec tone bursts were recorded in 8 normal subjects. Stimuli were presented at 60dB above hearing threshold to right and left ears separately. The P300 wave and cortical LAEPs were recorded over 450 msec from 16 scalp electrodes. Computerized four point linear interpolation was done to provide a spatiotemporal map of the EP, and by extension, a visualization of wave progression across the scalp. Subjects were all right handed, and ranged in age from 20 to 35.

Fourteen of the 16 AEPs showed a lateralization in the development and progression of the P300. Ten AEPs (5 right sided stimuli, 5 left sided stimuli) were lateralized in the right hemisphere during the P300 peak. Moreover, 6 of the latter 10 P300s were present in some part of the temporal lobe. These results suggest that P300 may have a temporal lobe origin. Studies in progress are reviewing whether aberrations in the lateralized P300 wavefronts can be seen in temporal lobe lesions.

486.20

P300 ASYMMETRIES IN SCHIZOPHRENIA ARE INDEPENDENT OF EEG REFERENCE SITE AND MEDICATION. S.F. Faux*, P.G. Nestor* & R.W. McCarley (SPON: J. Schildkraut). Dept. of Psychiat., Harvard Med. School/VAMC, Brockton, MA 02401

Previous auditory P300 topography studies from our group (e.g., Biol Psychiat, 23:776-790, 1988), using the Linked-ears EEG reference site (LER), have found a voltage deficit maximal at the left temporal region in chronic, medicated schizophrenics (SZ). Two experiments were performed to determine whether this finding was related to psychopathology or was an artifact of the possible effects of 1) LER, which theoretically may produce voltage distortions at the scalp; and 2) neuroleptic medications.

Experiment 1 compared P300 potentials ("oddball" paradigm) from 20 SZ with those from 20 age-matched normal controls (NL) using either LER or the nose reference (NR). Normalized P300 amplitudes (300-400 ms) at left/right central and temporal sites showed group-by-electrode site interactions for both LER ($F(4,35)=3.72$, $p<.05$) and NR ($F(4,35)=3.89$, $p<.05$), with SZ showing a left<right temporal P300. In an identical protocol, Experiment 2 compared P300 topography from 11 SZ (withdrawn from medication $\geq$14 days) with that from 11 NL. Group differences in normalized P300 distribution were again evident with SZ showing the same left-temporal deficit ($F(4,17)=2.96$, $p<.05$). These results strongly imply the presence of a pathological neurophysiological process in SZ because they rule out the possible confounds of previous studies.

486.21

IS POST-ICTAL ELECTRICAL SILENCE A PREDICTOR OF RESPONSE TO ELECTROCONVULSIVE THERAPY? T. Suppes, R. Gutierrez-Esteinou, H. G. Pope, Jr. Biological Psychiatry Laboratory, Laboratories for Psychiatric Research, McLean Hospital, Harvard Medical School, Belmont, Ma. 02178.

We hypothesized that post-ictal electrical silence (ES) on the electroencephalogram after electrically induced seizures might predict response of depression to electro-consulsive therapy (ECT). To test this hypothesis, twelve patients meeting DSM-III-R criteria for major depression were administered the Hamilton Depression Rating Scale (HDRS) prior to ECT and again after three and after six ECT treatments. A separate investigator, blind to the clinical status of the patients, rated the degree of ES obtained after each electrically induced seizure as "none," "partial," or "marked." A highly significant correlation was found between the average degree of electrical silence obtained after each of the first six seizures and the degree of improvement on the HDRS after six treatments (Spearman rank correlation coefficient = 0.79; $p < .001$). Partial correlations controlling for mean seizure duration and for basline HDRS showed even stronger associations between clinical improvement and degree of ES ($R = .82$, $p = .003$ and $R = 0.84$, $p = .002$, respectively). These findings suggest that the degree of ES following electrically induced seizures may represent a potential predictor of response to ECT. Possible mechanisms for this association will be considered.

486.22

INFORMATION PROCESSING AND DIABETES MELLITUS. J. Everett*[1], M.A. Kabene*[2], C. Harnois*[3], (SPON: A.R. Caggiula) School of Psychology[1,2] and Department of Ophthalmology[3], Laval University, Quebec City, Canada.

In order to assess the effect of Diabetes Mellitus on information processing capacities, event related potentials (N100 and P300) were measured on diabetics (N=78) and normals (N=52). Since it has been well documented that similarities between the retina and the brain exist, diabetics were divided into two groups: with (N=34) and without retinopathy (N=44), to assess the possibility that the possible brain impairment found in diabetics may occur in parallel with retinopathy. The subjects were required to pay attention to 15% of high pitch tones (2000Hz) and ignore 85% of low pitch tones (1000Hz) emitted randomly. The electrodes were placed at C_z (Active), A_1 (reference) and F_{pz} (ground). While no differences were found for N100 and P300 latencies, diabetics showed significantly smaller N1P2 and N2P3 amplitudes comparatively to controls. No differences were found between diabetics with or without retinopathy. These results seem to indicate selective attention deficits in diabetics regardless of their retinal status.

486.23

THE INFLUENCE OF EXOGENOUS CORTICOSTEROIDS ON EXPERIMENTAL HYPERTHERMIA/ RADIATION LESIONS IN DOG BRAIN. P.J. Hoopes*, J.A. DeLeo, R.W. Colburn*, D.W. Roberts*, and D.W. Coombs(SPON: F.E. Musiek). Dartmouth-Hitchcock Medical Center. Hanover, N.H.

Exogenous corticosteroids are essential for moderating many detrimental side effects associated with brain tumor treatment. However, they may also have an serious negative treatment effects, such as the sparing of tumor tissue (membrane stabilization) and a decreased immune response, which could otherwise result in more beneficial treatment. In the present study entailing 90 beagles, lesions of normal dog brain produced by interstitial hyperthermia and/or radiation treatment are being assessed. Based on clinical signs and MRI/CT imaging it was observed that therapeutically relevant doses of dexamethasone markedly altered the course of lesions. As expected, treatment reduce acute and subacute morbidity/mortility (intracranial edema, blood-brain-barrier breakdown and seizure activity). However, steroid treatment also markedly retarded normal brain tissue healing 30 days following lesioning (treatment) as evidenced by persistance of necrosis, and blood-brain-barrier breakdown. Morphologic and morphometric assessment of tissue, cellular and subcellular effects are currently being assessed radiologically and microscopically using non-enhanced and contrast-enhanced MRI/CT, immunohistochemistry, autoradiography and quantitative microcomputer imaging techniques. Supported by DHHS/NCI Grant CA 42604

486.24

NEW METHODS TO ESTIMATE THE NUMBER OF MOTOR UNITS IN A HUMAN HAND MUSCLE. J.F. Yang, R.B. Stein and J. Jhamandas. Division of Neuroscience, University of Alberta, Edmonton, Canada T6G 2S2

The number of motor units in the thenar muscle was estimated using 3 independent methods. The maximum surface electromyogram (EMG) and twitch response to stimulation of the median nerve was assumed to represent recruitment of all motor units in that muscle. The number of units was estimated by dividing the maximum response by the estimated single unit contribution. Single unit contributions to the EMG and force were obtained separately by spike triggered averaging (STA), intramuscular microstimulation (Mstim) and graded whole nerve stimulation. Approximately 20 units were used to estimate the average single unit size for each method, in each subject. The estimated number of units in 8 normal subjects ranged from 100 to 200 using both the Mstim and STA methods, in reasonable agreement with histological reports. The estimates based on whole nerve stimulation were more variable, ranging from 100 to 400. Results from 8 patients with cervical spinal cord injury suggested that some patients had normal numbers of motor units, while others had severe motor unit loss with corresponding enlargement of the surviving units. Both the STA and Mstim methods provided reasonable estimates in normals and were sufficiently sensitive to detect alterations in patients.
(Supported by MRC and AHFMR)

486.25

REFLEX CHARACTERISTICS OF SPINAL CORD INJURED (SCI) HUMANS. P.W. Nance, Dalhousie University, Halifax, Nova Scotia Canada B3H 4K4.

With a solenoid-driven hammer which delivers variable intensity of force from 1-10, the deep tendon reflex (DTR) force response curves were recorded for SCI & non-SCI subjects. Both groups show graded responses such that the greater the impact force the larger the DTR amplitude. There was a trend for the mean DTR of the SCI subjects to be **below** the mean of the non-SCI subjects for every impact intensity. Since it has been often observed that bladder distention worsens spasticity in SCI subjects, the H-reflex (H), vibratory inhibition (VI) and the DTR responses before and 30 minutes after bladder catheterization were studied in 8 SCI subjects. The average amount of urine obtained via catheterization was 125 cc. Overall, the H amplitudes and the VI were decreased after catheterization, but no change in the DTR responses were noted. In summary, the H and VI demonstrated neurophysiological change associated with bladder distention in SCI subjects which was not detected by the DTR. The results indicate that DTR "hyperreflexia" is not a feature of spasticity due to SCI.
Supported by the Canadian Paraplegic Association

486.26

INSTITUTIONAL REVIEW BOARD (IRB) EVALUATION OF NEUROSCIENCE PROTOCOLS. A.J.Popp and D.L.Moore*, Division of Neurosurgery, Albany Medical College, Albany, NY 12208

Institutional review of research involving humans is mandated by law and described in the Code of Federal Regulations (CFR). We analyzed our IRB's critiques of neuroscience protocols to identify areas of recurring difficulty for investigators.

Minutes for 96 monthly meetings were reviewed for the IRB's critique of all neuroscience protocols submitted during the first author's tenure as its chair (7/80-6/88). Criticisms recorded in the minutes were collated under the applicable CFR section, ultimately coalescing into four categories: procedural issues, protocol critique, consent critique, and patient rights.

111 (8%) of 1341 new protocols were neuroscience protocols: 29 were approved as submitted, 74 were approved with modifications, 5 were tabled for revision and subsequently approved, 3 were tabled and withdrawn. None were disapproved. A total of 244 individual criticisms were identified: procedural issues (N=54) (example: list investigator's phone number); protocol critique (N=28) (example: refine method of statistical analysis); consent critique (N=124) (example: clarify risk of research); patient rights (N=38) (example: state guarantee of confidentiality).

A majority (66%) of criticisms involved either patient rights or consent; most could have been avoided by fuller and more accurate disclosure to patients. "Procedural" criticisms (22%) could have been reduced by adherence to IRB requirements concerning technical aspects of protocol submission. A comparatively small number of protocol-design criticisms (11%) suggests either greater attention to this by investigators or less rigor in this aspect of the IRB's review.

TRANSPLANTATION: SPINAL CORD

487.1

EARLY CHANGES IN THE CONTROL OF PRIMARY AFFERENT EXCITABILITY FOLLOWING CONTUSION INJURY OF THE RODENT SPINAL CORD. F.J.Thompson, P.J. Reier, G.W. Schrimsher*, L. B. Jakeman, D. Winialski*., C. Lucas*, L. R. Ray*. Dept. of Neuroscience, University of Florida, Gainesville, FL. 32610.

Previous studies have demonstrated alterations of several measures of reflex excitability following spinal injury. To date, the mechanisms that underlie these changes have not been defined. In the present study we have examined electrophysiological changes precipitated by contusive injury of the adult rodent spinal cord. These lesions (25g/cm) were produced at the T8 level using a modification of the Allen weight drop method. At 6 or 28 days after injury, tests of low frequency depression of reflexes were conducted, along with other measures of reflex excitability.

Our results show that muscle afferent-elicited reflexes in contused animals were significantly ($p = .05$) more resistant to frequency depression than those elicited in intact controls. In intact animals, stimulus repetition at 1 Hz attenuated reflex amplitudes to 28% of control compared to 60% and 91%, respectively, in the 6 day and 28 day contused animals.

The mechanism responsible for low frequency attenuation of reflex amplitude with increased stimulus frequency has been argued to be presynaptic inhibition. Therefore, the resistance to frequency depression of reflexes in the present study, suggests that presynaptic inhibition was significantly reduced in the contused animals as compared with values obtained in intact controls. (Supported by NINCDS #NO1-NS-7-2300).

487.2

MAGNETIC RESONANCE IMAGING (MRI) OF FETAL CAT NEURAL TISSUE TRANSPLANTS IN THE ADULT CAT SPINAL CORD. E.D. Wirth III*, T.H. Mareci*, D.P. Theele*, S.A. Brown*, D.K. Anderson and P.J. Reier. Depts. of Neuroscience, Neurosurgery and Radiology, Univ. of Florida College of Medicine, Gainesville, FL 32610 and VA Medical Center, Cincinnati, Ohio.

MRI was evaluated for its potential as a diagnostic tool in determining the survival of fetal neural grafts in the adult cat spinal cord (SC). Three adult female cats received a hemisection at either the T11 or L2 level, followed immediately by implantation of either E37 fetal brainstem or neocortex, or E23 SC into the cavity. In a fourth cat a static load compresssion (i.e. contusion) lesion was made at the L2 level. Seven weeks post-injury, the lesion was resected and fetal brainstem tissue was similarly transplanted. The contused cat and two of the hemisected animals received oral cyclosporine (10 mg/kg) one day prior to transplantation and daily thereafter. Six months post-transplantation, the cats were anesthetized with Ketamine (3.2 mg/kg) and Xylazine (0.22 mg/kg) and maintained during imaging with Isoflurane (1% in 100% O_2). MRI was performed on a 2.0T SIS system with a curvilinear surface coil and the cat in the supine position. Multislice spin-echo images (TR/TE= 1000/30) were obtained in both the transverse and sagittal planes. The transplant site was first localized with 2 mm slices and then studied for detail using contiguous 1 mm slices. On these proton-density/T1 weighted images, the host SC and grafts could be readily distinguished. The graft in the contused cat appeared as a region of hypointensity within the SC, whereas the grafts in the hemisected cat SCs appeared as hyperintense areas. Two of the cats were perfused immediately following MRI and their SCs were removed. Gross inspection and light microscopy verified the presence of viable graft tissue, with the rostrocaudal and mediolateral extent of the transplants corresponding to the MR images obtained. Therefore, our findings indicate that MRI can accurately demonstrate the presence of very small regions of transplanted neural tissue in both the hemisected and contused spinal cord. MRI should prove to be a useful adjunct to anatomical, electrophysiological, and behavioral studies aimed at assessing graft-mediated functional repair of the injured spinal cord.

487.3

DEVELOPMENT OF FETAL CAT NEURAL GRAFTS IN ACUTE AND CHRONIC LESIONS OF THE ADULT CAT SPINAL CORD. D. K. Anderson, P. J. Reier, D. P. Theele, J. B. Munson, L. A. Ritz, S.A. Brown, and B. E. Zeller. VA Medical Center, Cincinnati, Ohio and Departments of Neurological Surgery and Neuroscience, University of Florida, Gainesville, Florida

We are assessing the development of intraspinal fetal neural tissue transplantated into the spinal cord (SC) of the adult cat. In 8 cats the SC was compressed at the L2 level. 7-9 weeks postinjury, the injury site was resected and either E23 or E37 fetal cat neocortex, or brainstem or SC was implanted into this cavity. In 5 cats, the SC was hemisected at L2 and similarily transplanted. Both sets of cats received oral cyclosporine (10 mg/kg) one day prior to transplantation and daily thereafter. At either 6 wks, 12 wks or 6 mo post-transplantation, the cats were perfuse-fixed and the SC examined by light and electron microscopy. Large grafts filled the lesion cavities and showed extensive vascularization without signs of necrosis or rejection. Cells in the neocortical grafts appeared immature and the surrounding neuropil was unmyelinated. Regions of close approximation were seen with no scar formation. The fetal SC grafts were large with histological features of the normal SC. These grafts were more mature and less intergrated with the host than the neocortical grafts. These findings indicate that intraspinal transplantation is feasible in acute and chronic lesions of the adult cat SC and that this model can be used to study functional repair of the injured SC.

487.4

REGENERATION OR SPROUTING OF CORTICOSPINAL TRACT AXONS INTO FETAL SPINAL CORD TRANSPLANTS IN THE ADULT RAT L.B. Jakeman and P.J. Reier. Departments of Neuroscience and Neurological Surgery. University of Florida College of Medicine. Gainesville, FL 32610.

If the corticospinal tract (CST) is lesioned in adult animals, the axons typically exhibit retrograde degeneration and retract from the site of the injury. Several studies from this laboratory have indicated that FSC transplants can provide a suitable environment for the growth or regeneration of other descending and local spinal cord axons, as well as primary afferent fibers. In a previous study, we used the anterograde transport of wheat germ agglutinin conjugated to horseradish peroxidase (WGA-HRP) to examine the response of injured adult CST axons in the presence and absence of FSC transplants. We found that in some cases when FSC tissue was directly apposed to the injured tract, labeled axons persisted at the host/graft interface. In addition, a limited number of CST axons appeared to have grown into the transplanted tissue. The current study has extended these findings by employing the anterograde tracer, *Phaseolus vulgaris* leucoagglutinin (PHA-L), to examine the projection patterns of individual CST axons in the presence of FSC transplants. Five adult rats (200-300 g.) received bilateral lesions of the dorsal funiculus and dorsal horn at the C_6 spinal level. A single piece of E_{14} FSC was then placed into the cavity. Fourteen weeks after grafting, three iontophoretic injections of 2.5% PHA-L (Vector) were made into each sensorimotor cortex. After allowing 20 days for transport of the tracer, the rats were perfused and sections stained immunocytochemically for the presence of PHA-L. In four recipients, labeled CST axons were observed within the transplants. The axons entered the grafts from the dorsal and lateral host-graft interfaces. Within the transplants, CST axons extended 0.5 - 1.0 mm, traveling in a circuitous pathway before branching around the donor cells. These findings clearly demonstrate that adult CST axons are capable of regeneration or sprouting after injury. Such regeneration is possible if the injured fibers are provided with a permissive environment such as developing spinal cord tissue. Supported by NS 22316, MH 15737.

487.5

ANALYSIS OF RATS RECEIVING FETAL SPINAL CORD TRANSPLANTS SUBSEQUENT TO MID-THORACIC CONTUSIVE SPINAL INJURY

D.L. Winialski and P.J. Reier (SPON: Wm. Friedman). Depts. of Neuroscience and Neurological Surgery, University of Florida College of Medicine, Gainesville, FL 32610.

We have previously reported evidence that fetal CNS grafts could repair sites of tissue damage in longstanding contusion injuries of the adult rat spinal cord. Only partial reconstruction was achieved, however, as cystic cavitation and dense astrocytic scarring were seen in some regions of host-graft approximation. In that study we exposed the cystic cavities and removed necrotic debris prior to implanting *solid* pieces of E_{14} spinal cord. In the present study we tested whether tissue repair could be enhanced by introducing *suspensions* of fetal spinal cord tissue into the epicenter of the contused spinal cord.

Using a modified Allen weight-drop apparatus, contusion injuries were made at the T_8 vertebral level. After post-injury delays of either 1d (N=22) or 1wk (N=7), dissociated E_{14} spinal cord cells (20 ul of vehicle per fetal spinal cord) were injected into the lesion epicenter. Typically, 4 injections of 5 ul each were made, yielding a total injection volume of 20 ul per recipient. After post-injury survival periods of 1-5 wks, animals were sacrificed, their spinal cords were embedded in plastic, and 2 um sections were examined. At 1wk post-implantation in either the 1d or 1wk post-injury recipients, relatively undifferentiated graft tissue surrounded by necrotic tissue and inflammatory cells was seen. However, at later times, well-myelinated grafts were observed which often filled the lesions and exhibited some degree of organotypic differentiation. Large patches of donor tissue were also often observed at levels immediately rostral and caudal to the epicenter where they were juxtaposed to host gray matter. Fusion of the grafts with host gray and white matter without intervening glial scarring was commonly observed.

These results demonstrate that successful transplantation of dissociated cells can be obtained as early as 24 hours after a contusion lesion. Additionally, the use of slurries of cells, rather than solid pieces, seems to enhance the quality of host-graft integration. [Supported by NINCDS NO1-NS-7-2300]

487.6

EFFECTS OF GABA ON NEURONS IN INTRAOCULAR SPINAL CORD GRAFTS J.G. Broton, R.P. Yezierski, and A. Seiger, Dept. Neurol. Surg., Univ. of Miami, Miami, FL 33136.

Gamma-amino-butyric acid (GABA) is present in the normal rat spinal cord. This study evaluated the effects of GABA administration on spontaneously-active spinal cord neurons which had developed without peripheral or supraspinal afferent input.

Grafts were taken from the lumbar spinal cord of E14 rat embryos and transplanted into the anterior eye chamber of adult rat hosts. After 5-8 mo., hosts were anesthetized with urethane and immobilized, the cornea cut, and a 10mm diam. chamber placed around the graft. The chamber held approximately 200ul of Earle's solution which was kept at 36°C by perfusion with warmed solution (rate=120ul/min).

The activity of 12 spontaneously-active (mean firing rate = 9.2 Hz) graft neurons was studied. Addition of 10ul of 30mM GABA into the recording chamber decreased the firing rate of all 12 neurons. The onset of this decrease varied from 1-27s (median=8.5s) and was not correlated with depth of the recording electrode below the graft surface. In 9/12 cases the neurons stopped firing for at least 10s after GABA superfusion. Administration of the GABA receptor antagonist bicuculline methiodide (1-2mM) before or after GABA increased the firing rate of all seven neurons tested.

These results suggest that GABA, not previously studied in intraocular spinal cord grafts, is present and capable of influencing the spontaneous activity of graft neurons. We are presently investigating the effects of GABA superfusion on intraocular co-grafts of spinal cord and mesencephalon in order to determine if the presence of target tissue changes the response of spinal cord graft neurons to GABA.

Supported by NS 19509 and by The Miami Project Foundation.

487.7

INTRAOCULAR SPINAL GRAFTS: LOCALIZATION OF NAAG, GLUTAMATE, AND GLUTAMATE DEHYDROGENASE. A.T. Salvatierra, Å. Seiger, and K.E. Miller. Dept. Neurol. Surgery, Univ. Miami, Miami, FL 33136; Dept. Geriatric Med., Huddinge Hosp., Huddinge, Sweden; G.D. Searle R&D, Monsanto Co, Chesterfield, MO 63198.

Intraocular grafting of neural tissue has been used as a model to study differentiation and growth regulation of CNS areas, including the spinal cord. In this study, grafts of fetal lumbar spinal cord were transplanted and maintained in oculo for several months. Host animals were anesthetized and perfused with fixative. Grafts were removed, frozen sectioned at 30µm, and processed for immunohistochemistry with antisera against N-acetylaspartylglutamate (NAAG), glutamate, and glutamate dehydrogenase (GDH). NAAG- and glutamate-immunoreactive (IR) neurons were found in most grafts. Some large neurons resembling motor neurons were NAAG-IR. GDH-IR puncta occurred throughout the grafts and GDH-IR astrocytes also were identified. The results of this study indicate that neurons and astrocytes that contain markers for cells involved in excitatory amino acid metabolism continue to develop and differentiate within intraocular grafts. The intraocular graft paradigm may be a useful model to investigate factors that regulate growth in excitatory amino acid neural systems. Supported by the Miami Project to Cure Paralysis.

487.8

TRANSPLANTATION OF EMBRYONIC BRAIN STEM INTO THE ADULT LESIONED BRAIN STEM. B.H.Hallas, H.F.Lowe, M.A.LaCorte, G.Jacobsen, S.D.Loughlin and S.P. Lee. New York College of Osteopathic Medicine, Wheatley Rd., Old Westbury, NY 11568

In adult host rats, a transverse scapel lesion was made through nucleus caudalis of the trigeminal brain stem nuclear complex at the level of the obex. Simultaneously, brain stems from fourteen-day rat embryos (E-14) were dissected, the lateral and caudal portion isolated, freed of meningeal membranes and transplanted into the lesioned area. Ninety days post-transplantation, HRP was injected into either the thalamus, superior colliculus, cerebellum, cervical spinal cord, rostral trigeminal subnuclei, somatosensory cortex, trigeminal ganglion, or into the transplant. Injections into the rostral trigeminal subnuclei and trigeminal ganglion showed that projections from these areas innervated the transplants. The other injection sites showed no label. Injections of HRP into the transplant demonstrated local efferents: i.e., connections with the adjacent trigeminal brain stem nuclei both contra and ipsilaterally and cervical spinal cord. In addition primary afferents rostral to the transplant were individually injected with HRP and total axonal boutons counted for each collateral that entered the transplant. Bouton counts for primary whisker and guard hair afferents did not significantly differ from normal. Supported by AOA grant #88-07-281.

487.9

INCREASED CATECHOLAMINE LEVELS IN SPINAL CORD SUPERFUSATES OF RATS WITH ADRENAL MEDULLARY IMPLANTS. J.E. Kemmler* and J. Sagen (SPON: M. Radulovacki). Dept. Anat. and Cell Biol., Univ. Il. at Chicago, Chicago, IL 60612.

The transplantation of adrenal medullary tissue into the subarachnoid space of the rat spinal cord can reduce pain sensitivity, particularly following stimulation by nicotine. This analgesia can be blocked by the opiate antagonist naloxone, and partially attenuated by the alpha- adrenergic antagonist phentolamine, suggesting that both opioid peptides and catecholamines (CAs) released from the implanted chromaffin cells mediate this effect. We have recently demonstrated that basal met- enkephalin levels in the spinal cord CSF of implanted animals are double that of control animals. The purpose of the present study was to assess changes in CA levels in the spinal cord CSF of these animals. Following determination of baseline pain sensitivities rats were implanted with adrenal medullary allografts in the spinal subarachnoid space at the lumbar enlargement. At various time intervals following implantation (2 weeks - 6 months), pain sensitivity was again assessed and CSF samples were collected from these animals using a spinal cord superfusion technique. Three 5 minute samples were collected to determine basal release, followed by nicotine injection and collection of an additional three samples. CAs were extracted by alumina, and levels determined using HPLC with electrochemical detection. Results indicated that the release of norepinephrine into spinal cord superfusates of implanted rats was nearly double that of control rats, even six months after implantation. In addition, epinephrine, which was barely detectable in the CSF of control rats, was increased at least 20-fold in implanted animals. Release of both of these CAs was further increased by nicotine injections. These results suggest that CAs are co-released with opioid peptides from implanted chromaffin cells, and further support the notion that the combined release of both CAs and opioid peptides contributes to the production of analgesia by spinal cord adrenal medullary implants. Supported by NIH grant NS25054.

487.10

PREVENTION OF LEARNED HELPLESSNESS BY MONOAMINERGIC NEURAL TRANSPLANTS TO THE RAT NEOCORTEX. C.E. Sortwell*, J. Sagen, and G.D. Pappas. Dept. Anat. and Cell Biol., Univ. Il at Chicago, Chicago, IL 60612.

The classic monoamine theory of depression states that depression may be caused by a central deficiency of norepinephrine and serotonin, and effective antidepressant and electroconvulsive shock (ECS) therapy work by correcting this deficiency. One of the most reliable and widely accepted animal models of depression is the learned helplessness (LH) model. The LH model has been shown to demonstrate good predictive validity for the treatment of human depression as it is reversed by antidepressant and ECS therapy. Previous work has demonstrated that the antidepressant desipramine prevents the development of LH in rats when injected directly into the frontal neocortex. Adrenal medullary chromaffin cells have been shown to release catecholamines, including norepinephrine, and the pineal gland has a very high concentration of brain serotonin. We transplanted either rat adrenal medullary tissue, rat pineal gland tissue, a combination of adrenal and pineal tissue, or equal volumes of control striated muscle into the frontal neocortex. Rats were trained for LH in a training box delivering cycles of uncontrollable shock for hour. Twenty-four hours later, LH was assessed in an escapable shuttle box. Depression (as measured by the LH model) was prevented in rats with adrenal medullary grafts, pineal grafts, and a combination of both adrenal and pineal grafts. In contrast, depression was not prevented by control striated muscle tissue grafts to the frontal neocortex. Morphological studies revealed that the grafted monoaminergic tissues survived well and continued to produce high levels of monoamines. These results suggest that neural transplants can provide a long-term source of monoamines for reducing depression.

487.11

FACTORS INFLUENCING CHROMAFFIN CELL SURVIVAL AND INTEGRATION IN THE CNS. J.D. Ortega*, J. Sagen, and G.D. Pappas (SPON: R.S. Cohen). Dept. of Anat. and Cell Biol., Univ. of IL at Chicago, Chicago IL 60612.

Previous work involving intraparenchymal xenografts of bovine chromaffin cells into the periaqueductal gray (PAG) of adult rats revealed that cell suspensions, in contrast to whole tissue implants, allow for greater host-graft integration. In an attempt to enhance survival and integration of the transplanted cells, rats were treated for various time intervals with several agents known for their ability to promote graft survival and enhancement of host-graft integration. These agents included nerve growth factor (NGF), ganglioside GM1, and the immunosuppressive agent cyclosporin A, administered alone or in combination. Isolated bovine chromaffin cells in primary culture were stereotaxically transplanted into the rat PAG in all cases. Animals were given daily injections of cyclosporin A for 3, 6, or 12 weeks. Perfusion for immunocytochemical or EM analysis was done either immediately following termination of drug treatment or delayed until the end of the 12 week period. Marked increased survivability was observed in all animals receiving cyclosporin A treatment, compared to untreated animals. In addition, it appeared that a short-term treatment with cyclosporin A was sufficient for this enhanced graft survival. NGF was continuously administered via an osmotic pump (2.5 S, 200 ug/ml, i.c.v.) either immediately following transplantation, or following a 2 week delay period. EM analysis of transplanted chromaffin cells in NGF treated animals were more pleomorphic than those of untreated animals, and appeared to extend fine processes towards neighboring host tissue. Increased numbers of synaptic contacts were also seen in NGF treated animals as early as 2 weeks post-implantation. Supported by NIH grants GM37326 and NS25054.

487.12

TOLERANCE STUDIES ON PAIN REDUCTION BY ADRENAL MEDULLARY IMPLANTS IN THE SPINAL CORD SUBARACHNOID SPACE. H. Wang* and J. Sagen. Dept. Anat. and Cell Biol., Univ. Il at Chicago, Chicago, IL, 60612.

Work in our laboratory has shown that pain sensitivity can be reduced by the transplantation of adrenal medullary chromaffin cells into the spinal cord subarachnoid space. The injection of low doses of nicotine produces potent analgesia in these animals. The analgesia most likely results from the stimulated release of opioid peptides from the transplanted cells, since it can be blocked by the opiate antagonist naloxone. Furthermore, the duration of analgesia following nicotine can be prolonged by kelatorphan, an enkephalinase inhibitor. Since a significant therapeutic problem with opiates is the development of tolerance, the purpose of this study was to determine the extent of tolerance development to the transplants. Dose-response relationships to several doses of nicotine (0.05 - 0.2 mg/kg, s.c.) and morphine (1.25 - 10 mg/kg, s.c.) were determined using analgesiometric tests prior to and following the transplantation of adrenal medullary or control tissue. In addition, some animals were implanted subcutaneously with constant release pellets containing several doses of nicotine (0.62 - 100 mg released over a three week period) and tested for dose- responsiveness to acute nicotine or morphine injections. Results indicated that adrenal medullary implants shifted the morphine dose- response curve to the left, suggested that, not only is there no cross tolerance to morphine, but the dose-responsiveness to morphine is actually potentiated by the adrenal medullary implants. Following implantation of nicotine pellets, dose-response curves to acute nicotine injections were shifted to the right by high pellet doses, while responses to morphine injections were unaltered. These results suggest that transplanted cell surface nicotinic receptors are less responsive when constantly stimulated by high doses of nicotine, but there is little tolerance at the host opiate receptor. (Supported by NIH grant NS25054).

LIMBIC SYSTEM II

488.1

A TIMM STAIN FOR ZINC WITHOUT SULFIDE PERFUSION M.D. Haigh*, C.J. Frederickson, G.A. Howell, (SPON: D.F. Johns) Lab for Neurobiology, Univ. of Texas/ Dallas, Richardson, TX. 75083

A previously described post-mortem Timm method (Chafetz, 1986, Brain Res. Bull.) has been modified to produce reliable zinc staining patterns in the brain that are consistent with traditional Neo-Timm's histochemistry. Specific modifications include (1) increasing the concentration of Na_2S from 0.37% to 2%, (2) changing from a phosphate buffer to TRIS, (3) soaking slides in Na_2S for 30 sec rather than dipping slides repeatedly as described in the previously published method. We believe that a key factor in our results has been to scrupulously acid clean all slides and glassware to remove any metallic contamination.

The zinc staining patterns that are obtained with this method are directly comparable to those obtained by perfusion with sodium sulfide in that the hippocampal sub-fields can be differentiated (including the inner and outer molecular zones of the fascia dentata) and cell bodies are unstained. The methodological improvements result in a post-mortem sulfide stain that is a useful addition to the study of zinc histochemistry in the brain, with potential applications in the study of a variety of human pathological conditions.

488.2

TSQ FLUORESCENCE SURVEY OF ZINC-CONTAINING BOUTON DENSITIES C.J. Frederickson, B.A. Rampy*, S. Remy Rampy*, G.A. Howell, Lab for Neurobiology Univ. Texas/Dallas, Richardson, TX. 75083

Timm-Danscher histochemistry shows that many limbic and cerebrocortical regions are innervated by metal-containing axonal boutons. The fluorescent marker for zinc, TSQ (Molecular Probes), indicates that zinc is the metal in most of these regions. In this work, we used ZN:TSQ fluorescence to estimate the relative abundance of zinc-containing boutons in 19 rat brain regions. Frozen 20 um sections were stained with TSQ, and fluorescence (500 nm; 55 um sampling spot) was measured microspectro-photometrically. Compared to the Zn:TSQ fluorescence from the zinc-rich hilus of the dentate gyrus, structures such as the lateral amygdala (fluorescence (i) = 40% of hilus) and subiculum (i=25%) showed relatively high fluorescence. Neocortical laminae II-III (i = 21%) and V (i = 13%), and the neostriatum (i = 7%) were also fluorescent, whereas structures that are essentially unstained by Timm-Danscher methods gave readings near zero (corpus callosum = 1.9%, neocerebellar cortex = 0.9%). The data emphasize that zinc-containing fiber systems are abundant throughout the forebrain.

488.3

TOPOGRAPHY AND SYNAPTOLOGY OF MAMILLOTEGMENTAL PROJECTIONS IN THE RAT. G.V. Allen and D.A. Hopkins. Dept. Of Anatomy, Dalhousie University, Halifax, N.S., Canada, B3H 4H7.

The mamillotegmental tract (MTG) is a major descending pathway for conveying information from the mamillary body (MB) and other limbic system structures to the brain stem. Although previous studies have described the broad outlines of descending projections from the MB (Guillery,'57; Cruce,'77), very little is known about the fine structural organization of MTG endings in the midbrain tegmentum (Takeuchi et al.,'85). In the present study, the distribution and synaptic organization of MTG fibers in the midbrain tegmentum were analysed following injections of WGA-HRP into the MB. The brains were sectioned and reacted for HRP reaction product in tetramethyl benzidine (TMB), diaminobenzidine (DAB), TMB stabilized with DAB, or TMB stabilized with ammonium molybdate and processed for light and electron microscopy.

After injections of tracer into the lateral and medial mamillary nuclei, dense anterograde and retrograde labeling were observed in the dorsal and ventral tegmental nuclei, respectively, and dense topographically organized anterograde labeling was observed extending from the medial portion of the nucleus reticularis tegmenti pontis into the rostral medial pontine nuclei. At the electron microscopic level, labeled axon terminals were observed in the dorsal and ventral tegmental nuclei and the nucleus reticularis tegmenti pontis and medial pontine nuclei. The labeled terminals were small (diameter <2 μm), contained mainly round synaptic vesicles and formed mainly asymmetric synaptic junctions with small diameter dendritic profiles and occasionally with neuronal somata. In addition, labeled terminals were found in synaptic contact with labeled dendrites and neuronal somata in the dorsal and ventral tegmental nuclei. The presence of labeled terminals on labeled postsynaptic elements in the midbrain indicates that cells which project to the MB receive direct reciprocal inputs from the MB. In addition, the present results indicate that mamillotegmental terminations in the midbrain are located primarily on distal dendrites and are most likely excitatory in nature. Supported by MRC of Canada.

488.4

GUDDEN'S TEGMENTAL NUCLEI ARE THE MAJOR SOURCE OF GABA FOUND IN THE MAMMILLARY BODY OF THE RAT. T.R. Stratford and D. Wirtshafter. Department of Psychology, University of Illinois at Chicago, Box 4348, Chicago, IL 60680

The mammillary body (MB) of the rat contains a significant amount of gamma-aminobutyric acid (GABA), most of which appears to be localized in fibers and terminals. It is well known that the MB possesses strong reciprocal connections with the ventral (VTN) and dorsal (DTN) tegmental nuclei of Gudden, both of which have been shown to contain substantial populations of GABAergic neurons. The possibility that these nuclei are the source of GABA found in the MB was investigated by the selective lesioning of the VTN or DTN combined with the immunocytochemical demonstration of the GABA synthesizing enzyme glutamic acid decarboxylase (GAD) using the Oertel - Kopin primary antibody. Lesions of the DTN selectively depleted GAD levels in the ipsilateral lateral mammillary nucleus (LM). Lesions of the VTN resulted in a comparable depletion of GAD in the LM as well as depletion of the ipsilateral premammillary nucleus (PM) and the lateral division of the medial mammillary nucleus (MM). Neither lesion significantly affected GAD levels in the medial division of the MM. These results suggest that the principal GABAergic input to the LM arises in the DTN and that the ascending DTN fibers pass near the VTN en route to the LM. Additionally, GABAergic efferents from the VTN appear to be the primary source of GABA found in the PM and lateral division of the MM. (Supported by NS-21350)

488.5

THE NUCLEUS ACCUMBENS AND THE ORGANIZATION OF BEHAVIORAL SEQUENCES IN MONKEYS (*MACACA FASCICULARIS*). C.E. Stern* and R.E. Passingham* (SPON: M.E. Hasselmo). Dept. of Experimental Psychology, Univ. of Oxford, S. Parks Rd., Oxford, OX1 4JF

Nucleus accumbens lesions impair the organization of behavioral sequences such as hoarding in rats (Kelley & Stinus, 1985, Behav. Neurosci. 99:531). The following experiments explore the role of this region in primate behavior.

A comparison was made between 6 unoperated control monkeys (*Macaca fascicularis*) and 6 monkeys which received ibotenic acid lesions of the Nucleus Accumbens (NA). The NA lesioned monkeys were significantly impaired on a hoarding task in which they were required to remove 18 peanuts from their shells. The control animals removed the nuts from the shells and subsequently stored them in their cheek pouches (nuts eaten = 4.7%, nuts dropped = 7.4%). The NA lesioned animals, which were able to pick up and store the nuts when they were presented without shells, dropped 22.5% of the nuts and ate 41.7% of the nuts without storing them when they were presented in shells. This disruption does not appear to be related to general changes in motivation, as the NA lesioned monkeys were willing to work for nuts on a button press task (900 button presses in under 10 minutes for 60 peanuts).

A hypothesis that the hoarding impairment resulted from an inability to sequence the necessary behaviors (pick up, crack, peel, and store) is substantiated by data obtained on a 4-box search task. Pre-operatively, the monkeys were taught to retrieve 4 food rewards from four identical boxes with lids. The control animals became very consistent in their behavior, opening the boxes from right to left (box 1 to 4) the majority of the time. In contrast, the NA lesioned animals became less consistent in their use of the right to left strategy used by controls ($T=4.1$, $df=4$, $p<.05$) and returned to previously opened boxes more often than the controls ($T=5.7$, $df=4$, $p<.01$). These results suggest that the NA may play a role in the organization of behavioral sequences in primates.

488.6

ELECTROPHYSIOLOGICAL EVIDENCE FOR RECIPROCAL AND TOPOGRAPHIC PROJECTIONS BETWEEN THE NUCLEUS ACCUMBENS (NAS) AND VENTRAL PALLIDUM (VP). R. L. Hakan, G. Berg* and S.J. Henriksen. Research Inst. of the Scripps Clinic, La Jolla, CA 92037.

Although a number of neuroanatomical studies suggest projections from the NAS to the VP, attempts to antidromically invade NAS single units by VP stimulation have indicated only a small percentage (~10%) of NAS neurons as VP projection cells. Additionally, recent neuroanatomical reports have suggested that the VP reciprocally projects back to the NAS. As part of ongoing electrophysiological studies of the NAS in anesthetized rats, we have observed that the NAS does project to the VP and that the VP reciprocally projects back to the NAS. Analyzing the antidromic and monosynaptic orthodromic effects of VP stimulation it is also apparent that these projections show a significant medial-lateral topography within the NAS such that NAS-VP projecting cells are found in the lateral NAS while VP-NAS projection cells are found predominantly in the medial NAS. Further evidence of this reciprocal circuitry was also found in subsequent experiments showing both orthodromic and antidromic responses in cells recorded within the VP following stimulation of the NAS. The concurrent analysis of VP and fimbria input to the NAS suggests that these inputs interact, possibly in a monosynaptic convergence upon the same NAS neuron. Further analysis of these interactions should help elucidate NAS integration processes. (Supported by DA03665 and KO2/DA00131 to S.J.H.)

488.7

HIPPOCAMPAL PROJECTIONS TO RAT NUCLEUS ACCUMBENS: A LIGHT AND ELECTRON MICROSCOPIC ANALYSIS USING PHA-L. T.L. Quirk and P.M. Groves. University of California, San Diego. La Jolla, CA 92093.

A variety of evidence exists which suggests that the nucleus accumbens may be involved in the integration of limbic and motor activity. The accumbens receives cortical inputs from allocortical areas, particularly the hippocampal region, and projects to regions associated with motor behavior such as the mesolimbic dopamine system and the mesencephalic locomotor region. The hippocampal projections to nucleus accumbens have not been extensively characterized, particularly at the ultrastructural level. The present study employed injections of the anterograde tracer *Phaseolus vulgaris* leucoagglutinin (PHA-L) into the dorsal subiculum in order to label hippocampal afferents to nucleus accumbens. Male Sprague-Dawley rats were anesthetized with pentobarbital and stereotaxically injected with a 2.5% solution of PHA-L (5-7μA, 15 min.). Following a survival period of 7-14 days, animals were deeply anesthetized with pentobarbital and perfused transcardially. For light microscopic studies, animals were perfused using a PH shift protocol. Sections were incubated in a 1:2000 dilution of rabbit anti-PHAL antibody for 48 hours and then processed for immunoperoxidase labeling. In accord with the observations of Groenewegen et al. (Neuroscience, vol. 23 (1), pp. 103-120, 1987), subicular afferents to nucleus accumbens exhibit a topographical distribution such that anterior, dorsal regions of subiculum project to more rostral and lateral zones in accumbens while more posterior and ventral parts of subiculum innervate progressively more caudal and medial regions of accumbens. Subicular fibers exhibit various types of morphology. The most commonly observed type of fiber is thin and set with irregularly spaced, small varicosities. These fibers are most often found in the central and lateral aspects of the accumbens. Along the mediodorsal borders of the nucleus accumbens, PHA-L labeled axons occur which are thicker and exhibit a "curly" type of morphology. In some cases these fibers are studded with large, closely spaced varicosities and also give off occasional terminal boutons; in other cases they are smoother and lack terminal specializations. For electron microscopic studies, injected animals were perfused with an acrolein-paraformaldehyde protocol. Sections were processed for immunocytochemistry using either immunoperoxidase or silver-intensified colloidal gold procedures. Labeled regions of interest were identified under the light microscope and are currently being processed for electron microscopy.

488.8

Mesencephalic and Supramammillary Projections to the Hippocampal Complex in the Rat R.P. Dilts and J.F. McGinty Dept. of Anatomy and Cell Biology, East Carolina University School of Medicine Greenville, NC 27858

The hippocampus receives a projection from the A8-A10 dopamine region of the ventral mesencephalon which extends into the supramammillary region of the posterior hypothalamus. Injections of the retrograde tracer fluorogold (FG) were made into the temporal CA1 region of the hippocampal complex. These injections were combined with fluorescent immunocytochemistry for tyrosine hydroxylase (TH) and cholecystokinin (CCK) in an attempt to localize the source of the dopaminergic projection to the hippocampal complex. The retrogradely labeled cells observed within the A8-A10 dopamine region were limited in their distribution to the ventral most portion of the nucleus paranigralis of the ventral tegmental area. These labeled neurons increased in number rostrally forming a continuum into the supramammillary region. Most labeled neurons were found medioventral to the A10 dopamine region, with few labeled cells observed within the TH-positive regions. An occasional cell was observed to stain positively for TH and FG fluorescence. Likewise, a few CCK immunoreactive cells contained retrograde label. However, the majority of retrogradely labeled cells within this region did not stain positively for TH or CCK. We are exploring the identity of other neurotransmitters which may be located within this pathway. Supported by DA 03982

488.9

PHA-L-TRACING OF THALAMIC AFFERENTS TO THE HIPPOCAMPUS AND PARAHIPPOCAMPAL REGION. LIGHT AND ELECTRON MICROSCOPY. F.G. Wouterlood, M.P. Witter* and E. Saldana*. Dept. Anat., Vrije Univ., Amsterdam, The Netherlands, and Dept. Morphol. Sci., Univ. Sch. of Medicine, Salamanca, Spain.

We injected *Phaseolus vulgaris*-leucoagglutinin (PHA-L) in the nucleus reuniens thalami of the rat to study the projection of this nucleus to the hippocampus and parahippocampal areas. At the LM-level, we combined the tracing with immunocytochemistry of GABA, vasoactive intestinal polypeptide (VIP), and neuropeptide-Y (NPY). There is dense homogeneous labeling in the stratum lacunosum-moleculare of the entire hippocampal field CA1, the molecular layer of the dorsal and ventral subiculum, layer I of the para- and presubiculum, layers I, III and IV of the dorsolateral entorhinal cortex (DLEA), layers III-V of the ventrolateral entorhinal cortex, layer I of the perirhinal cortex, and layers III-IV of the medial entorhinal cortex (MEA). The labeling is clustered in layer I of the MEA and the caudal part of the DLEA. GABA-immunoreactive cell bodies in the areas of termination are frequently apposed by PHA-L-labeled fibers, whereas cell bodies immunoreactive for VIP or NPY are not. At the EM level, the axon terminals of the thalamohippocampal fibers form asymmetric synaptic contacts with dendritic spines and thin shafts of spinous dendrites and contain spherical synaptic vesicles.

488.10

THE LATERAL MAGNOCELLULAR NUCLEUS OF THE RABBIT R.W. Sikes and B.A. Vogt Dept. of Physical Therapy, Northeastern Univ. and Dept. Anatomy Boston Univ. Medical School, Boston, MA 02115.

The lateral magnocellular nucleus of Gerhardt (LM) contains the largest neurons in the rabbit thalamus. The neurons of LM are predominantly large, oval and darkly staining although a limited number of smaller, lightly staining neurons are present. The large neurons have a very high cytochrome oxidase activity.

In order to identify the cortical projections of this nucleus, injections of horseradish-peroxidase and fluorescent dyes were placed into the cortex. Labeled neurons were observed primarily after injections into medial area 29d although some cells were labeled by injections into area 29c. Only caudal injections into area 24 resulted in labeling of LM neurons. A crude topography in the projection to area 29d was revealed by multiple injections of fluorescent dyes. The medial limb of LM projects rostrally while the lateral limb projects to the most caudal extent of area 29d. These results are the first to demonstrate that LM provides a major thalamic input to cingulate cortex in rabbits.

488.11

FUNCTIONAL PROJECTIONS FROM THE RAT HIPPOCAMPAL FORMATION TO THE MEDIAL FRONTAL CORTEX: AN *IN VIVO* INTRACELLULAR STUDY. T. D. White, A. M. Tan*, and D. M. Finch. Department of Neurology and Brain Research Institute, University of California, Los Angeles, CA 90024

Recordings of long latency (often > 20 ms) inhibitory (47%) and excitatory (4%) post-synaptic potentials were obtained from principal neurons (N=69) in medial frontal cortex (MFC) in response to stimulation of the hippocampal formation (HF). Yet, principal neurons (N=61) in the HF were frequently (59%) antidromically activated by stimulation of the MFC. These results indicate that a significant oligosynaptic projection from the HF provides feedforward inhibition to the MFC. In addition, five candidate inhibitory neurons (CIN) were identified in MFC which responded to stimulation of the HF. One class of CIN (N=4) exhibited short duration bursts of action potentials followed by inhibition. A second class of CIN (N=1) responded with a long duration burst of action potentials without subsequent inhibition. The excitatory bursts of the first class of CIN corresponded to the Cl-dependent inhibitory post-synaptic potentials of principal cells. The excitatory burst of the second class of CIN corresponded to the non Cl-dependent inhibitory postsynaptic potentials of principal neurons and to the duration of inhibition in the first class of CIN. A model of MFC circuitry is presented. Supported by NIH Grant NS 16721.

488.12

FUNCTIONAL PROJECTIONS FROM THE RAT MEDIAL FRONTAL CORTEX TO THE ENTORHINAL CORTEX AND SUBICULAR COMPLEX: AN *IN VIVO* INTRACELLULAR STUDY. D. M. Finch, A. M. Tan*, and T. D. White. Department of Neurology and Brain Research Institute, University of California, Los Angeles, CA 90024

A number of recent anatomical studies have shown extensive projections from medial cortex to the entorhinal cortex and subicular complex. Since there are virtually no cellular physiological studies of these pathways, we studied them using *in vivo* neurophysiological recording techniques. Sprague-Dawley albino rats (N=110) were anesthetized with chloral hydrate (400 mg/kg, i.p., supplemented by i.m. injections as necessary), and stimulating and recording electrodes were lowered stereotaxically to their targets.

A high proportion of neurons within the medial frontal cortex were antidromically activated at short latency (<1 msec) by electrical stimulation of the entorhinal cortex or subicular complex, indicating fast direct projections from the medial frontal cortex. The predominant synaptic response in entorhinal or subicular complex neurons after electrical stimulation of the medial frontal cortex was inhibition, as shown by the presence of Cl-dependent IPSPs. A small percentage of entorhinal cortex and subicular complex principal cells showed clear EPSPs. Candidate inhibitory cells were also encountered. The results indicate the presence of a physiologically significant cortico-cortical association pathway between these two distant regions. Supported by NIH Grant NS 16721.

488.13

AMYGDALOID PROJECTIONS TO THE PREFRONTAL CORTEX IN THE RHESUS MONKEY. H. Barbas and J. De Olmos*. Boston Univ. and Sch. Med., Boston, MA 02215, and Inst. Invest. Med., Cordoba, Argentina.

Projections from the amygdala to **basoventral** (orbital periallocortex, proisocortex, areas 13, 11, 12 and ventral 46) and **mediodorsal** (areas 25, 32, 14 and 8) prefrontal cortices were studied with retrograde tracers (horseradish peroxidase and fluorescent dyes). The orbital limbic areas (periallocortex and proisocortex) had the strongest links with amygdaloid nuclei, including the basolateral, basomedial (also known as accessory basal), lateral and to a lesser extent the cortical. Mediodorsal prefrontal areas received projections mainly from the basomedial and basolateral nuclei. Medial limbic area 25 and adjacent area 14 received a higher proportion of their projections from the basomedial than from the basolateral nuclei. The most architectonically differentiated basoventral or mediodorsal cortices (areas 46 and 8) had fewer connections with the amygdala, and these originated exclusively from the basolateral nucleus.

The results indicate that orbital limbic, followed by medial cortices, have widespread connections with several amygdaloid nuclei, whereas the most differentiated prefrontal cortices have few and topographically restricted amygdaloid connections.
(Supported by NIH grant NS24760).

488.14

LIMBIC INNERVATION OF THE NEOCORTEX: LAMINAR AND AREAL DISTRIBUTION OF THE PROJECTIONS FROM THE AMYGDALA IN THE CAT. A. Llamas*, F. Clascá* and F. Reinoso-Suárez. Departamento de Morfología, Facultad de Medcina, Universidad Autónoma, 28029 Madrid, SPAIN.

In an attempt to investigate limbic influence on the cerebral cortex, we have studied monosynaptic connections from the amygdala (A) to the entire neocortex of adult cats. Seventy-six animals received injections of HRP in discrete sectors covering the entire neocortical mantle. Twelve further animals received small deposits of WGA-HRP in A trough a micropipette. Our results indicate that axons from magnocellular basal amygdaloid nucleus (ABMc) innervate a vast extent of the neocortex, encompassing prefrontal, premotor, and motor areas, insular and perirrinal cortices, somatosensory areas SII and SIV, as well as ventral sectors of SI, auditory area AII, sectors of the suprasylvian fringe, and visual areas Ps and 20. Rostral limbic areas and retrosplenial cortex were densely innervated, while area cingularis (24) was almost free of amygdaloid connections. ABMc axons selectively reached the deep part of layer I, layer VI or both in most of those areas, but also layers II-III in the premotor, insular an perirhinal cortices. Lateral amygdaloid nucleus projects to a much reduced field, including caudal insular cortex, perirrinal areas, ventral sectors of temporal association auditory fields as well as infralimbic and ventral prefrontal areas. Laminar arrange of AL projections was rather uniform. These results provide an anatomical basis for selective monosynaptic input from the limbic system over a wide array of neocortical regions involved in sensory, association or motor processing. *CAICYT Grant PB86-0558 and FISSS 87/162 (F.C.)*

488.15

RESPONSES OF AMYGDALOID CENTRAL NUCLEUS (ACE) NEURONS TO STIMULATION OF THE PARABRACHIAL NUCLEUS (PBN) IN RABBITS. J.P. Pascoe and B.S. Kapp. Department of Psychology, The University of Vermont, Burlington, VT 05405.

The ACE is one essential component of a forebrain system that contributes to cardiovascular activity during emorional arousal. The PBN has reciprocal connections with the ACE and may be an important relay in both ascending and descending pathways between the ACE and lower brainstem cardioregulatory nuclei. We are examining activity in ACE neurons during sensory stimulation and following single pulse stimulation of the PBN using our standard methods.

Activity in 71/127 ACE neurons was infrequent (<.03 Hz) and was not increased to sensory stimuli. Of these, 19 were activated antidromically at latencies of 5-45 ms (25 +/-14 ms), and 24 were activated synaptically at latencies of 8-60 ms (26+/-18 ms). Activity in 15 other slowly discharging neurons (1 Hz) was increased to sensory stimuli (143 ms), and 6 of these were activated synaptically at latencies of 8-60 ms (24+/-17 ms). Activity in 32 rapidly discharging neurons (19.3 Hz) was increased (86 ms, n=15), decreased (86 ms, n=10), or unchanged (n=7) to sensory stimuli, and PBN stimulation increased (n=19) or decreased (n=3) activity in 22 of these at latencies of 5-40 ms (17 +/-8 ms). Thus, activity in ACE efferents to the PBN is similar to that of other ACE-brainstem efferents described previously (Pascoe & Kapp, 1987), and stimulation of the PBN often increases activity in ACE neurons. Supported by the American Heart Assn and the AHA Vermont Affiliate, Inc.

488.16

ANTICONFLICT EFFECTS OF CHLORDIAZEPOXIDE FOLLOWING MULTIPLE LESIONS OF THE AMYGDALA. H. Grishkat*, C. Strickland*, AND E. Yadin. Department of Psychology, Bryn Mawr College, Bryn Mawr, PA 19010.

Rats were trained in a Vogel conflict paradigm in which unpunished water drinking was permitted for 2 min followed by a 2 min punished drinking segment. This sequence was repeated for a total session time of 8 min. The onset of the punished segments was signalled by a tone, indicating that every twentieth lick would be accompanied by a mild footshock (0.25-0.6 mA). A stable baseline rate of punished licking was established by titrating shock levels so that punished licking was 10-40% of unpunished lick rates. The number of licks was recorded. Animals underwent bilateral electrolytic lesioning of the central, basolateral, and medial nuclei of the amygdala. Two weeks after surgery, animals were retested in conflict.

Punished drinking increased significantly postoperatively. After adjusting shock levels to regain the pre-op baseline response levels, chlordiazepoxide (2.5-10.0 mg/kg i.p.) was administered. A dose dependent increase in punished responding was found. These results suggest that the amygdaloid nuclei are not necessary for the anxiolytic effects of the benzodiazepines to be expressed.

HIPPOCAMPUS AND AMYGDALA II

489.1

METALLOTHIONEIN INDUCTION IN RAT HIPPOCAMPAL NEURONS IN PRIMARY CULTURE. P. Thakran[1]*, M.P. Leuschen[1], M. Ebadi[2]. Depts. of Pediatrics[1] and Pharmacology[2]. Univ. of Neb. Med. Ctr., Omaha, NE.

Primary cultures of neurons are important tools for the study of environmental influences on neuronal expression. Our interest is in the role of trace elements in epileptogenesis and the influence of metal binding proteins in temporal lobe epilepsy. The induction of metallothionein, a low molecular weight, cysteine-rich protein believed to regulate the flow of essential trace elements through cellular compartments, has been studied in cell lines and tissues other than the brain. We have developed a model system for culturing hippocampal neurons in the cysteine-free Iscove's modification of Dulbecco's MEM (IMDM) supplemented with 25 mM K^+, 30mM D-glucose, 293 mg/l L-glutamine, 100 mU/l insulin, 7μM p-amino benzoic acid, 100 μg/ml transferrin, 10 mg/ml BSA, 10^{-12} M β-estradiol, 100 μg/ml gentamicin and 3 μg/ml fungizone. Two culture substrates were utilized: Poly-L-Lysine and Collagen (derivatized to plastic culture dishes by a cross-linking reagent Carbodiimide). Poly-L-Lysine proved superior to Collagen as a culture substrate since neurons explanted at a comparatively later stage of development (60 days post-natal) showed earlier neurite outgrowth (within 3-5 days in vitro) as compared to those from neonatal rats plated on collagen. Neurons were identified by histochemical staining for cholinesterase. All cultures survived for 3 weeks and induced metallothionein synthesis in response to zinc (at concentrations of 10^{-8} to 10^{-6} M) as judged by ^{35}S-cysteine incorporation. Maximum induction occurred after 48 hrs. incubation with zinc.

489.2

EFFECT OF ADRENALECTOMY (ADX) ON DISTRIBUTION OF BASIC FGF (FGF) mRNA IN THE HIPPOCAMPUS. A. Baird*, S.A. Frautschy*, A. M. Gonzalez* and J. Farris* (SPON: R. Guillemin) Molecular and Cellular Growth Biology, Whittier Institute, 9894 Genesee La Jolla, CA 92037

In the brain basic FGF has been shown to have neurotropic action both *in vivo* and *in vitro*. We have previously shown with *in situ* hybridization that basic FGF mRNA is distributed in neurons localized primarily in the CA2 region of the hippocampus. Since this region densely concentrates steroids such as glucocorticoids, the purpose of this experiment was to investigate the effect of ADX on distribution of basic FGF mRNA in the hippocampus. Male adult rats were adrenalectomized, sham adrenalectomized or given corticosterone (B) replacement +adrenalectomized. Distribution of basic FGF mRNA was examined using *in situ* hybridization one week after surgery. Animals were perfused with paraformaldehyde using the pH shift method. 25-μm sections were mounted on poly-lysine-coated slides. The rat basic FGF clone RO basic FGF was subcloned into pBluescript SK+ and anti-sense probe was transcribed using T7 RNA polymerase and 35S-UTP. The upstream sense strand was used as a control. After hybridization, sections were treated with ribonuclease A and washed in 0.1 SSC at 55°C. Slides were exposed to Kodak NTB-2 autoradiograph emulsion for 4 weeks. Results showed that basic FGF mRNA was distributed in the hippocampus of control, adrenalectomized and adrenalectomized+B-treated rats primarily in the CA2 region. This agrees with our previous results in 90-day-old male rats. Strong signaling was also observed in the CA1 region. In summary, it appears that short-term ADX has no effect on hippocampal distribution of basic FGF mRNA. An effect of long-term ADX on distribution of basic FGF mRNA cannot be excluded.

489.3

Expression of SNAP-25 Protein and mRNA Following Hippocampal Lesions. J.W. Geddes, E.J. Hess, R.A. Hart*, J.P. Kesslak, C.W. Cotman, and M.C. Wilson. *Univ. Calif., Irvine CA 92717, and Scripps Clinic and Res. Foundation, La Jolla CA 92037.*

A neuronal-specific mRNA, expressed at high levels in CA3 pyramidal neurons of the mouse hippocampus (MuBr8, Branks and Wilson, *Mol. Brain Res.* 1, 1-16, 1986), encodes a novel 25 Kd synaptosomal protein (SNAP-25) that potentially contains a zinc binding domain (Oyler et al., submitted). We examined the expression of SNAP-25 protein and mRNA in the rat hippocampal formation, using immunochemistry and *in situ* hybridization, following hippocampal injection of kainic acid or colchicine and following electrolytic lesions of the entorhinal cortex. Kainic acid lesions, which destroy pyramidal CA3 and hilar neurons, did not alter the pattern of SNAP-25 immunoreactivity in CA3 but did result in decreased immunoreactivity in the inner 1/3 of the molecular layer of the dentate gyrus. Colchicine, which selectively removes the granule cells of the dentate gyrus and the mossy fiber projection to CA3, abolished SNAP-25 immunoreactivity in CA3. Following electrolytic lesions of the entorhinal cortex, SNAP-25 immunoreactivity in the inner molecular layer was intensified and expanded to occupy the inner 1/2 of this region, consistent with a sprouting of the commissural/associational system which originates in the hilar neurons. These results were similar to those observed using Timm's stain for zinc and demonstrate that SNAP-25 is located within the presynaptic terminals of the mossy fibers and of the mossy cell projections to the molecular layer of the dentate gyrus. SNAP-25 is a novel presynaptic marker of select hippocampal pathways that may be useful in studies of neurological disorders such as Alzheimer's disease.

Supported by NIA LEAD Award and by NIH Grant NS2303A.

489.4

HIPPOCAMPAL CA1 AND CA3 DIFFERENCES IN VULNERABILITY AFTER FOUR-VESSEL OCCLUSION (4-VO) ISCHEMIA IN THE RAT J.M. Ordy, P. Colombo*, J. White*, E. Cregan*, G. Thomas, B. Volpe, and W. Dunlap*, Fisons Pharmaceuticals, Rochester, NY 14623, Cornell Med. Center, New York, NY 10021, and Tulane Univ., New Orleans, LA 70118

Patients with global cerebral ischemia have circumscribed memory loss and selective hippocampal CA1 damage. In the rat (4-VO) model of stroke, memory loss and CA1 damage occur after 30 min. of ischemia. In hippocampal memory circuits, sensory information flows from the entorhinal cortex through perforant path, dentate gyrus, CA3, CA1, subiculum, to entorhinal cortex. CA3 neurons provide major intra-hippocampal integration and CA1 neurons major efferent hippocampal information output. The aims of this study were to compare differential CA1-CA3 cell vulnerability after 30 min. of 4-VO ischemia. Damage was evaluated in 6 μM sections, stained with H & E, 14 days after 4-VO. BMDP2V programs were used to compare CA1-CA3 cell damage. Compared to controls, 4-VO ischemia produced highly significant CA1 ($p < 0.001$) and lesser but significant CA3 ($p < 0.01$) cell damage. Damage in CA1 was significantly ($p < 0.01$) greater than in CA3. Gradients of hippocampal cell vulnerability depended upon duration of 4-VO ischemia, vascular, and cellular factors. Studies have been undertaken to determine the extent to which ischemically compromised but viable CA1-CA3 neurons may be amenable to drug modification in stroke.

489.5

COMMISSURAL PROJECTIONS IN THE NEONATAL RAT HIPPOCAMPUS. J.R. Buchhalter, A.W. Fieles* and M.A. Dichter. Dept. Neurology, Univ. of Pennsylvania, Philadelphia, PA., 19104.

We investigated hippocampal commissural projections in the neonatal rat using retrogradely transported rhodamine labelled microspheres (RLM).

Sprague-Dawley rat pups (ages 3-5 days) were injected with 0.1-0.5 ul RLM into the right hippocampus. 48 hours was allowed for retrograde transport. Pups were then sacrificed, brains fixed in 4% paraformaldehyde and placed in a 20% sucrose solution. Brains were frozen in isopentane and immersed in liquid nitrogen prior to sectioning on a freezing microtome. 30 u sections were examined with a fluorescence microscope to determine site of injection and contralateral transport.

In 3 of 4 CA1 injections (each injection represents one brain), label was found in the homotopic contralateral CA1. In 1 CA1 injection, contralateral label was present only in CA3. All 5 injections into CA3 produced contralateral CA3 labelling. In 5 brains, injections were made into CA1 and CA3. Of these, contralateral label was found in the indicated frequency: CA3 (1), CA1 and CA3 (2), CA3 and the dentate hilus (DH) (1), DH only (1). CA1 and DH were injected in 2 brains and produced contralateral homototopic labelling. Control injections into the ventricular system did not label either hippocampus. When the commissure was cut prior to CA1 injection, no label appeared in the contralateral hippocampus.

These results suggest that hippocampal commissural projections exist in the neonate, but the CA3 to CA1 connection present in the adult was not demonstrated. Further, a CA1 to CA1 projection was noted. The latter pathway has not been consistenly reported in the adult. This work was supported by NINCDS grant # 02150.

489.6

ANTEROGRADE TRACING WITH BIOCYTIN DEMONSTRATES A PRECISE CORRESPONDENCE BETWEEN THE DENTATE GYRUS INNER MOLECULAR LAYER TIMM'S STAIN BAND AND THE HILAR AFFERENT PROJECTION FIELD. P. M. Louis, M. A. King, B. E. Hunter, and D. W. Walker, VA Medical Ctr. and Department of Neuroscience, University of Florida, Gainesville, FL 32610.

The Timm's sulfide-silver technique reveals the location of certain metals in brain tissue, for example the 3 bands in the molecular layer (ML) of the dentate gyrus. Although these bands are generally considered to represent the synaptic terminal fields of particular dentate afferents, this idea has never been explicitly tested. We used biocytin to anterogradely label hilar afferents to the dentate ML in brains that were also stained by the Timm's method. Adult male Long-Evans and Wistar rats were anesthetized with Nembutal and burr holes drilled in the skull. Biocytin was pressure ejected into the dorsal dentate hilus. Rats were overdosed with Nembutal 1-4 days postinjection and perfused using the Timm's fixation protocol. Postfixed brains were Vibratome sectioned at 40 microns and sections reacted with avidinylated peroxidase or alkaline phosphatase followed by their respective chromogens. Precise correspondence was always observed between the inner ML Timm's stain band and the anterogradely labelled hilar axons and terminals, confirming that this Timm's band is a reliable marker for the hilar afferent terminal zone in the inner ML. Supported by the Veterans Administration and NIAAA grant A00200.

489.7

RECURRENT MOSSY FIBER COLLATERALS IN THE HUMAN HIPPOCAMPUS: A QUANTITATIVE STUDY. C. Thalmann*, H.-P. Lipp, D.P. Wolfer* and U. Zollinger*. Institute of Anatomy and Institute of Forensic Medicine, University of Zürich, Switzerland.

Hippocampal mossy fibers emit collaterals (MFC) which dynamically innervate the granule cell layer (CGL) and a supragranular zone (SGL) of the fascia dentata. Lesions in rats have been reportet to cause adult sprouting. In guinea pigs, the MFC show defined growth spurts around puberty and in the midlife period (*Wolfer and Lipp, Soc. Neurosci.Abstr. 14:891,1988*). In aged human brains, exuberant growth has been reported (*Cassell & Brown, J.Comp. Neurol. 222:461, 1984*). An excessive growth seems also to be associated with epilepsy (*Sutula et al., Science 239: 1147,1988*). For any comparison, however, it is necessary to know the area and distribution of MFC at different ages. Here we report the first results of a systematic quantification.

Nineteen hippocampi (age range 14-90 years, no documented neurological problems) were sectioned in a parasagittal plane along the unco-septal axis. Every 10th cryostat section (40µ, unfixed) was analyzed (12-14 sections per case). The area of each section covered by Timm-stainable boutons was seperatly determined for CGL and SGL by means of videometry in 30 sampling areas along the unco-septal axis.

As shown in rats and mice, there is a distinct unco-septal gradient of MFC (uncal >> septal). Post mortem time correlated moderately with the amount of Timm-stainable MFC, indicating the need for a correction factor. Yet, most striking was an enormous interindividual variability (coefficient of variation in CGL: 55%, in SGL: 192%). The area covered by MFC appeared completely *unrelated to age* (r=0.125, n.s.). No gender differences were found.

Thus, we have been unable to confirm previous reports of age-dependent growth of MFC in humans. Also, any comparison with brains from defined patient populations ought to be based on rather large samples in order to avoid misleading conclusions. Supported by Swiss National Science Foundation (SNF 3 .206-0.88).

489.8

MORPHOLOGY, DISTRIBUTION AND ULTRASTRUCTURE OF SEROTONERGIC AXONS IN THE HIPPOCAMPAL FORMATION OF THE CAT. I. Törk, T. Orfanos* and J.-P. Hornung. School of Anatomy, University of New South Wales, Kensington, NSW 2033, Sydney, Australia.

The serotonergic axons were demonstrated in horizontal sections of the hippocampal formation using a monoclonal antibody against serotonin (Sera-Lab, U.K.), in combination with the biotin-avidin peroxidase technique. Three types of labeled axons were observed: thick, non-varicose fibres, fibres with small (<1µm), fusiform varicosities, and fibres with large (2-7µm) varicosities. The density of the fibres in the different cytoarchitectonic regions of the hippocampal formation was measured using a semi-automatic computerized system, and density profiles of every region were created. A highly characteristic pattern of the distribution of serotonergic axons was observed: in the hippocampus, the highest density was measured in the CA3 region, and in the stratum lacunosum-moleculare. There was remarkably high density in the subiculum, with relative paucity of fibres in the adjacent pre- and parasubiculum and CA1 regions. In the dentate gyrus the density of fibers was relatively low, except for a thin layer of axons subjacent to the granule cell layers. We have also studied the synaptic connections the serotonergic axons made. The large varicose axons made asymmetrical connections with neurons found in the hilus of the dentate gyrus and subiculum, while the axons with small varicosities formed synaptic connections infrequently.

The present results strongly suggest that the serotonergic system has a highly specific and differential role in the modulation of nervous activity in the hippocampal formation. The observations also extend recent reports regarding the dual organization of the serotonergic fibre system in the cortex. (Mulligan and Törk, J. Comp. Neurol. 270: 86,1988; Mamounas and Molliver, Exp. Neurol. 102: 23, 1988).

Supported by a grant from the National Health and Medical Research Council of Australia.

489.9

CHARACTERIZATION OF THE EXTRINSIC EFFERENT PROJECTIONS OF NON-PYRAMIDAL NEURONS IN THE HIPPOCAMPUS. Th. van Groen and J.M. Wyss. Dept. of Cell Biol. and Anat., Univ. of Alabama at Birmingham, Birmingham, AL 35294.

Most research into the extrinsic projections of the hippocampus proper in the rat has focused on the contribution of pyramidal neurons in area CA_1, and relatively little attention has been given to the potential contribution of non-pyramidal neurons to these projections. Recent studies in our laboratory indicated that the hippocampal projections to the retrosplenial cortex originate partly in these so-called "interneurons". To further investigate the projections of these non-pyramidal neurons, retrograde tracing studies were conducted; injections of fast blue and fluorogold were made into the regions to which the hippocampus projects. Injections into cortical areas (i.e. the retrosplenial, entorhinal, and the pre- and parasubicular cortices) labeled non-pyramidal cells that were at the border of stratum moleculare and radiatum in both area CA_3 and CA_1. Each of these injections labeled neurons in the pyramidal cell layer of area CA_1 that were relatively confined to a small region of the septotemporal axis of the hippocampus. In contrast, the labeled non-pyramidal neurons were distributed widely along the septotemporal axis of the hippocampus. Injections into several subcortical areas, e.g. the nucleus accumbens, the lateral septal nucleus and the horizontal and vertical limb of the diagonal band of Broca, also retrogradely labeled neurons in the hippocampus, but most of the non-pyramidal neurons labeled by these injections were in stratum radiatum and oriens in area CA1 and the subiculum, and the labeled non-pyramidal neurons were confined to those regions on the septotemporal axis that contained labeled pyramidal neurons. These results demonstrate that the putatively inhibitory interneurons of the hippocampal formation project outside of the hippocampal formation and that different groups of putative interneurons project to different areas.

489.10

TRAJECTORY OF COMMISSURAL FIBERS FROM ENTORHINAL CORTEX IN THE CAT. Donald Siwek* and Bertram Payne (SPON: M. Feldman). Department of Anatomy, Boston University School of Medicine, Boston, MA 02118.

The purpose of the present study was to determine the trajectory of axons in the hippocampal commissures of the cat. In other species, the hippocampal commissures convey axons between the entorhinal, presubicular and hippocampal regions of the two hemispheres, and between the hippocampal formation of one side and the septal nuclei of the contralateral side. With this knowledge in mind, three injections of either tritiated amino acids or horseradish peroxidase were made into entorhinal cortex of two cats to reveal the trajectory of hippocampal commissural axons. Cells and axons in entorhinal cortex along the full length of the parahippocampal gyrus and part of the adjacent hippocampal formation were exposed to the labels. Commissural axons leave entorhinal cortex and course through the alveus and fimbria to run forwards and medially in the crus of the fornix. Along their course the axons aggregate into fascicles, and a number of the fascicles cross the midline caudally in the dorsal hippocampal commissure, which lies anterior to the splenium of the corpus callosum. At more rostral positions other fascicles cross the midline obliquely in the hippocampal decussation. The majority of fascicles continue rostralwards to the body of the fornix where they then cross to the opposite side in the ventral hippocampal commissure. After crossing the midline fascicles retrace the same pathway, except at a mirror image position, to their site of termination in contralateral structures. Additional observations made of 2µm thick plastic embedded sections and thin sections examined with the electron microscope show that the fascicles are poorly myelinated, and many of the constituent axons have no myelin sheaths at all. (Supported by EY06080 and EY06404)

489.11

NADPH-DIAPHORASE HISTOCHEMICAL PROFILES IN HIPPOCAMPAL FORMATION OF SQUIRREL MONKEY. D.R. Brady, R. Carey & E.J. Mufson (SPON: P.Riely). Inst. Biogerontol. Res., Sun City, AZ 85351. NADPH-diaphorase (NADPH-D) histochemistry specifically labels a subset of topographically organized neurons and fibers in the hippocampal formation (HF) and entorhinal cortex (EC) of squirrel monkey. Sections through HF and EC were histochemically reacted for NADPH-D, with selected sections counterstained for Nissl substance to aid architectural delineations. NADPH-D positive (NADPH-D+) neurons were pleomorphic, exhibiting multipolar, pyramidal, bipolar or small oval somata and long dendritic arbors. NADPH-D histochemistry revealed many large, densely stained neurons and a population of small, lightly stained neurons. The larger neurons were found in the alveus of HF, the white matter of Arnold's bundle and the deep layers of EC. Lightly labeled NADPH-D+ neurons occupied the superficial layers of EC. NADPH-D+ fiber staining was greatest in HF at rostral levels, diminishing in intensity caudally. NADPH-D+ fibers in medial EC were oriented radially with bilaminar bands of heavy reactivity in layer 1 and subjacent white matter. NADPH-D+ reactivity in lateral EC was trilaminar (heavy in layers 1, 4 and white matter) and superimposed on a dense plexus of radially arranged NADPH-D+ fibers. The distribution and morphology of NADPH-D stained neurons in monkey HF and EC revealed an interesting pattern of reactivity that may be related to the functional architecture of this pivotal medial temporal cortex. Supported by NS 26146 and Arizona Disease Control Research Commission.

489.12

THE MONKEY HIPPOCAMPAL FORMATION RECEIVES INPUTS FROM AREAS 35, 36, TF AND TH BUT NOT FROM AREA TE. W.A. SUZUKI*, R. INSAUSTI AND D. G. AMARAL The Salk Institute, La Jolla, CA. 92037 and Dept. of Anatomy, Univ. Navarra, Spain.

The hippocampal formation receives most of its cortical sensory information from polysensory associational areas in the frontal, cingulate and temporal cortices through projections that terminate in the entorhinal cortex (Insausti et al., J. Comp. Neurol. 264:356-395, 1987). It has recently been suggested that the unimodal, visual area TE has direct, reciprocal connections with field CA1 of the hippocampus (Yukie and Iwai, Neurosci. Letts, 88: 6-10, 1988; Brain Res. 444:397-401, 1988). The extent to which unimodal associational areas project directly to the hippocampal formation will undoubtedly influence conceptualization of the role of the hippocampal formation in memory function. To study the connectivity of field CA1, we injected retrograde tracers into the CA1/subiculum border of the hippocampal formation in 5 Macaca fascicularis monkeys. In the inferior temporal lobe, most of the labeled cells were found in the medial half of both the perirhinal (areas 35 and 36) and parahippocampal (areas TF and TH) cortices; smaller numbers of labeled cells were observed in the lateral half of these fields. No labeled cells were observed in area TE. Conversely, while ^{3}H-amino acid injections into the perirhinal and parahippocampal cortices resulted in anterograde labeling of the CA1/subiculum border region, an injection placed into area TE did not lead to such labeling. Our anatomical data therefore indicate that the CA1 field, like the entorhinal cortex, receives primarily polymodal sensory input.

489.13

ORGANIZATION OF AMYGDALOID PROJECTIONS TO VISUAL AREAS OF THE OCCIPITAL AND TEMPORAL LOBES IN THE MONKEY. D. G. Amaral and F. Nahm* The Salk Institute, La Jolla, CA. 92037.

In previous anterograde tracing studies (Amaral and Price, 1981) direct projections were demonstrated from the amygdaloid complex to much of the visually-related neocortex. To determine the location and topographic organization of the cells of origin for these projections, injections of the fluorescent retrograde tracers Fast Blue and Diamidino Yellow were made into different regions of the occipital and temporal lobes in 8 Macaca fascicularis monkeys. Histological sections through the amygdaloid complex were surveyed for the occurrence of single or double labeled neurons and their positions were plotted using a computer-aided digitizing system. Injections located in cortical regions ranging from area 17 caudally to anterior area TE rostrally resulted in labeled cells in the amygdala. The largest number of retrogradely labeled cells was observed in the basal nucleus mainly in its dorsal or magnocellular region. The rostrocaudal focus of labeled cells in the basal nucleus was related to the rostrocaudal position of the injection site in the temporal or occipital lobes. When injections were placed rostrally and ventromedially in the inferotemporal cortex, labeled cells were also observed in the accessory basal nucleus. Few doubled labeled cells were observed in these experiments even when the two injections were separated by as little as 3 mm. These studies confirm that the basal nucleus projects widely to visual regions of the temporal and occipital cortices and that individual cortical regions appear to be innervated by separate populations of amygdaloid neurons.

489.14

MORPHOLOGICAL CHARACTERISTICS OF MEDULLARY PROJECTION NEURONS IN THE RAT CENTRAL AMYGDALOID NUCLEUS. C-J. Shi*, L. Modaressi* and M.D. Cassell* (Spon. T.C. Ritchie), Dept. of Anatomy, Univ. of Iowa, Iowa City, IA 52242.

The central amygdaloid nucleus (Ce) contain five cytoarchitectonic zones, each containing specific cell types. Previous studies have demonstrated, that the distribution pattern of neurons project to the dorsal medulla overlaps several of these cytoarchitectonic zones. This suggests the presence of multiple forms of Ce neurons projecting to the medulla. To attempt to identify the morphologies of Ce projection neurons, we have used a combined retrograde HRP tracing technique with the section Golgi technique. Twelve adult male rats received injections of WGA-HRP into the rostral part of the nucleus of the solitary tract. Following histochemical reaction with DAB (Streit and Reubli, 1977), the sections were treated with 0.1% Os 0_4 + 3.5% K_2 Cr_20_7 overnight, sandwiched between two glass coverslips, and put into 1.5% Ag $N0_3$ solution. After drawings and photographs were taken, the silver impregnation was dissolved with 1~2% $Na_2S_20_3$ and the sections examined to identify Golgi cells additionally labeled by HRP. The results demonstrate that there are two basic types of brainstem projection neurons distributed in the medial part of Ce; a medium-sized pyramiform neuron with long, rarely branching dendrites with a low to moderate spine density; and a bipolar type with few, if any spines. Previous studies had suggested that the bipolar type may have had only local connections. Projection neurons distributed in the lateral and ventral parts of Ce resembled the pyramiform type of medial Ce neuron. Projection neurons located in the substantia innominata were also of the medial Ce pyramiform type. These findings suggest that projections from the Ce to the dorsal medulla arise from similar cell types, irrespective of their cytoarchitectonic location. (Supported by NS 25139).

489.15

Topographical organization of insular cortex projections to the rat central amygdaloid nucleus. M.D. Cassell* and L. Modarressi.* (Spon. A.K. Afifi). Dept. Anat., Univ. of Iowa, Iowa City, IA 52242.

Recent studies (e.g., Cechetto and Saper, 1987) have divided the rat insular cortex into a rostroventral dysgranular zone and caudodorsal granular zone corresponding to special and general visceral sensory representations respectively. Several studies have reported projections to the central amygdaloid nucleus (Ce) from the insular cortex (IC) but there has been little information on the specific termination patterns of projections arising from specific insular regions. Accordingly, 25 adult Sprague-Dawley rats received small, iontophoretic injections of 1.5% HRP-WGA into specific regions of the insular cortex. Injection placements varied rostrocaudally by ± 3 mm relative to bregma and dorsoventrally by ± 2 mm relative to the fundus of the rhinal sulcus. To confirm that the anterograde HRP labelling observed in the Ce following TMB histochemistry was not due to labelling of collaterals of retrogradely labelled neurons, small electrolytic lesions were made in the IC and anterograde degeneration subsequently detected using a modified Fink-Heimer technique. Examination of the terminal patterns produced by both techniques revealed that rostral and ventral parts of IC (corresponding to dysgranular and agranular regions) innervate the lateral subdivision of Ce whereas the dorsal and posterior IC (the granular zone) innervates the medial subdivision. Lesions/injections involving the most caudal IC and adjacent perirhinal cortex produced terminal labelling over the lateral capsular subdivision of Ce. These findings suggest a differential representation of visceral information in the Ce that possibly relates to the distribution of Ce neurons projecting to the parabrachial complex and dorsal medulla. These are located respectively in lateral and medial parts of Ce.

Supported by NIH grant NS25139.

489.16

IN VIVO CHARACTERIZATION OF AMYGDALAR PHYSIOLOGICAL RESPONSES TO BASAL FOREBRAIN AND HIPPOCAMPAL COMPLEX STIMULATION IN RATS. L. E. Mello*, A. M. Tan* and D. M. Finch (SPON: M. Nuwer). Department of Neurology and Brain Research Institute, University of California, Los Angeles, CA 90024

Amygdaloid responses to electrical stimulation of the basal forebrain and hippocampal complex were recorded *in vivo* from chloral hydrate anesthetized adult male Sprague-Dawley rats. Recordings were usually carried out with 60-90 MΩ micropipets filled with 1M K-citrate. In some animals, a 1M KCl pipet was used to study the Cl^- dependence of IPSPs. A few cells were injected with HRP (5-10% in 0.05 M Tris-KCl, pH 7.6) to correlate physiology and morphology.

Responses usually consisted of antidromic activation, followed by an EPSP, action potential, and IPSP. Some of the animals showed synaptic responses with latencies shorter than 4.0 ms, suggesting monosynaptic activation. The morphology of most injected cells corresponded to the Class I neurons of McDonald [J. Comp. Neurol. 212 (1982) 293-312]. We were able to invert some of the IPSPs evoked by basal forebrain stimulation using KCl pipets, indicating that these IPSPs were, at least partially, mediated by Cl^- influx. A few cells fired a burst of action potentials with a latency slightly shorter than the IPSP latency of neighboring cells, suggesting that they were inhibitory cells. Supported by NIH Grants NS 23074 and NS 16721 and CNPq (Brazil).

489.17

THE EFFERENT CONNECTIONS OF THE AMYGDALO-HIPPOCAMPAL AREA: A *PHASEOLUS VULGARIS* LEUCOAGGLUTININ TRACT-TRACING STUDY IN THE RAT. N.S. Canteras, R.B. Simerly† and L.W. Swanson†, Neural Systems Lab, The Salk Institute & †Howard Hughes Medical Institute, La Jolla, CA 92037.

The amygdalo-hippocampal area (AHZ) lies between the caudal pole of the medial amygdala and the ventral subiculum and appears to be a major site for hormonal feedback on neural functions. The AHZ contains many cells that concentrate gonadal steroid hormones (Pfaff and Keiner, '73; Sar and Stumpf, '74), as well as cells that express high levels of mRNA encoding mineralocorticoid, estrogen, and androgen receptors (Arriza et al., '88; Simerly et al., Soc. Neurosci. Abstr., '89). However, a complete understanding of the functional role of the AHZ will require the identification of possible routes along which this humoral information may be transmitted. Although the projections of the ventral subiculum and the medial amygdala have been clearly defined, the specific connections of the AHZ have received little attention in the literature. In order to clarify the efferent projections of the AHZ we used the anterogradely transported lectin *Phaseolus vulgaris* leucoagglutinin (PHA-L). Iontophoretic injections of PHA-L were made into the region of the AHZ in over 30 animals and the brains processed as described elsewhere (Gerfen and Sawchenko, '84).

Like the ventral subiculum, the AHZ projects to the ventral part of the lateral septal nucleus (LSv). However,taken as a whole the projections of the AHZ show a marked similarity to the projections of the posterior dorsal part of the medial nucleus of the amygdala (MeAp). Following injections of PHA-L that were nearly entirely localized to the AHZ, and did not label cells in the MeAp, a dense plexus of labeled fibers and terminals was found in the MeAp. A compact bundle of labeled fibers coursed through the stria terminalis and provided a massive input to the encapsulated part of the bed nucleus of the stria terminalis, located just ventral and posterior to the LSv. The remainder of these fibers passed ventrally into the hypothalamus, and labeled fibers were found in nuclei within the periventricular and medial zones of the hypothalamus. The strongest projections were to those hypothalamic nuclei that are known to contain the highest densities of gonadal steroid concentrating cells, including the anteroventral periventricular nucleus, the medial and central subdivisions of the medial preoptic nucleus, the ventrolateral part of the ventromedial nucleus, and the ventral premammillary nucleus. The results of experiments in which injections of the retrograde tracer Fluorogold were made into each of these nuclei have confirmed these connections.

489.18

EXCITATORY AMINO ACID IMMUNOHISTOCHEMISTRY DISTINGUISHES BASOLATERAL AMYGDALOID NEURONS PROJECTING TO THE PREFRONTAL CORTEX VERSUS MEDIODORSAL THALAMIC NUCLEUS. A. J. McDonald, Department of Anatomy, Univ. of South Carolina Sch. of Med., Columbia, SC 29208.

Injections of WGA-HRP were made into the lateral and medial prefrontal cortex (PFC; n=3) or mediodorsal thalamic nucleus (MD; n=6) in 9 rats. Brains were processed for HRP-TMB histochemistry with DAB-cobalt stabilization followed by avidin-biotin immunohistochemistry for glutamate (GLU) or aspartate (ASP). Numerous immunostained GLU+ and ASP+ neurons in the basolateral amygdala (BLA) were retrogradely labeled by PFC injections (preliminary experiments suggest almost 100% colocalization of GLU and ASP in BLA). In contrast, no GLU+ or ASP+ neurons were labeled by MD injections. These findings support the results of previous studies in this laboratory which indicated that BLA projections to the two poles of the MD-PFC system originate from two distinct neuronal subpopulations. The absence of GLU and ASP immunoreactivity in BLA neurons projecting to MD suggests that these neurons do not use excitatory amino acids as neurotransmitters. The high levels of GLU and ASP in BLA neurons projecting to the PFC suggests that these neurons might use GLU, ASP, or a related substance as a neurotransmitter. (Supported by NIH Grant NS 19733).

489.19

THE HUMAN AMYGDALA: DEFINITION AND QUANTITATION OF NUCLEAR REGIONS. C.E. Byrum, J. de Olmos, and H.R. Brashear, University of Virginia Department of Neurology, Charlottesville, VA 22908.

The amygdala is a heterogeneous limbic structure that exhibits significant pathological involvement in Alzheimer's Disease (AD). At least nine distinct nuclei have been described which differ in their anatomical connections. However, parcellation of the amygdala into nuclei is difficult, and has been based on Nissl staining. Work in this lab has shown that the distribution of neuritic plaques in the amygdala in AD is nonuniform among nuclei, suggesting involvement of the amygdala may be related to specific anatomical connections. However, that study relied on sampling of areas of each nucleus because many borders were difficult to define. The present study was undertaken to define the normal anatomy of the amygdala for quantitative comparison in aging and neurodegenerative diseases. Five postmortem human brains were obtained from neurologically normal patients. Wet-mounted sections were photographed to produce sharp images of fiber tracts within the amygdala, then stained for Nissl substance to study cytoarchitectural features. Adjacent sections were processed for AChE histochemistry. The combination of these methods yielded an accurate parcellation of the amygdala. Volumetric measurements of each nucleus of the amygdala were then obtained. Initial measurements of nuclear volumes in the normal human amygdala revealed: lateral n. 33%, basolateral n. 25%, basomedial n. 15%, cortical n. and cortical transition areas 10%, central n. 3%, medial n. 2%. Preliminary examination of postmortem brains from AD patients reveals loss of AChE staining and atrophy of the entire amygdala that is more pronounced in certain nuclei. These methods will facilitate determination of the distribution of immunohistochemical markers, pathological lesions, and atrophy in the human amygdala. Supported by NIH HDO7323 and AG00407.

489.20

IDENTIFICATION OF NON-PYRAMIDAL CELLS IN THE ADULT HUMAN HIPPOCAMPUS BY MEANS OF ANTI-PARVALBUMIN IMMUNOSTAINING E. Braak, B. Strotkamp, H. Braak Dept. Anatomy, J.W. Goethe University. D-6000 Frankfurt/M. 70, FRG

The pattern of cellular processes seen in Golgi impregnations is used for classification of neurons. Golgi impregnations of adult human autopsy specimens are rarely successful. Therefore the dendritic arborization pattern was previously correlated with the lipofuscin pigment pattern. Within the hippocampus nonpyramidal cells are either almost devoid of lipofuscin or contain numerous lipofuscin granules. By means of monoclonal anti-parvalbumin serum (1) numerous nonpyramidal cells in the adult human hippocampus were visualized (chromogene 4-chloro-1-naphthol (CN)). Documentation was followed by bleaching CN and a pigment-Nissl staining. Remarkable are 1) large horizontal fusiform neurons in the stratum oriens; they are strongly pigmented in sector CA 3 and non-pigmented in CA 1, 2) multipolar neurons in the stratum pyramidale in CA 1 giving rise to dendrites extending into the molecular layer and being either heavily or non-pigmented, 3) double-bouquet cells in the molecular layer of CA 1 being aligned parallel to the layer and always non-pigmented. The immunostaining with anti-parvalbumin depicts non-pyramidal cell types in the adult human hippocampus in an almost Golgi-like manner. These neurons resemble Golgi impregnated neurons in young animals. (1) Celio,M.R. et al. Cell Calcium 9: 81-86, 1988 Supported by the Deutsche Forschungsgemeinschaft.

HIPPOCAMPUS AND AMYGDALA III

490.1

SIGMA RECEPTOR LIGAND D-PENTAZOCINE (PENT) INCREASES FIELD POTENTIAL AMPLITUDES IN THE HIPPOCAMPAL FORMATION. C.U. Eccles, A.E. Cole, J.J. Aryanpur* and R.S. Fisher. Dept. of Neurol., Johns Hopkins Hospital, Baltimore, MD 21205 and Dept. of Pharm. and Toxicol., Univ. of Maryland School of Pharmacy, Baltimore, MD 21201.

Selective ligands for the sigma receptor have been developed but agonist/antagonist interactions for these ligands and a physiological role for this receptor have yet to be elucidated. The aim of this study was to test the hypothesis that a high affinity ligand such as PENT can influence the physiology of hippocampal neurons in regions containing high densities of sigma receptors. Field potentials evoked by orthodromic stimulation were recorded in stratum pyramidale of the CA1 region of the rat hippocampal slice. Bath application of PENT (5 uM, n=5 slices) increased field potential amplitude an average of 150-200% over a range of stimulus intensities (30-100V). The effect of PENT was not influenced by naloxone or the N-methyl-d-aspartate antagonist amino-phosphonovaleric acid (n=5) and could be blocked by the high affinity sigma ligand haloperidol (n=3). A moderate increase in amplitude was also measured in orthodromically-evoked field potentials recorded from the granule cell region of the dentate gyrus (n=3). These results indicate that a low concentration of PENT alters the physiology of hippocampal neurons and that the effect may be mediated via the sigma receptor.

490.2

SPONTANEOUS EEG SPIKE CORRELATES OF NEURONAL DISCHARGE IN THE CA3 REGION OF RAT HIPPOCAMPUS. M. Oguri and M. Kaneko*, Department of Biometrics, Faculty of Science, Toho University, Tokyo, Japan.

Highly conspicuous spikes are superimposed on spontaneous hippocampal EEG activity during behaviors not accompanied by rhythmical slow activity such as awake immobility and slow wave sleep. This study determines whether state-related EEG spikes share characteristics of discharge manifested by CA3 neurons. Movable macroelectrodes were placed in the CA1 region of rat hippocampus, together with bundles of fine-wire microelectrodes for recording CA3 neuronal discharge and electrodes to record eye movements, nuchal EMG, diaphragmatic EMG, and cortical EEG. EEG spike and CA3 unit activity were recorded in intact, unrestrained, drug-free rats, and were processed by statistical methods of power spectrum density, Markov dependency, and cross-correlation. No analyses were performed on REM sleep data, as EEG spikes were replaced by theta waves in that state. Discharge frequency in both EEG spikes and neuronal discharge showed similar patterns during each sleep-waking state. EEG spike occurrences were frequently associated with enhanced CA3 neuronal discharge.

490.3

THE TETRODE: AN IMPROVED TECHNIQUE FOR MULTI-UNIT EXTRACELLULAR RECORDING M.L. Recce and J. O'Keefe Department of Anatomy and Developmental Biology, University College London, London, England, WC1E 6BT. (SPON: BRA)

Extracellular recording in freely moving animals is a powerful technique for the study of brain function. In the hippocampus it has led to the discovery of place-coded neurones and indirectly to the cognitive map theory of hippocampal function. Difficulties with the present techniques limit their usefulness. To achieve stable recordings it is necessary to use insulated 25µm microwires with flat tips. While these electrodes overcome the stability problem they give relatively poor isolation of single units in densely packed cell layers. The recording range of the electrode includes electrical potentials from numerous cells, many of which are equidistant from the tip. This places serious limitations on the ability to unravel the spatial and temporal coding of environmental information within the hippocampus. Our previous solution to this problem involved the use of a paired set of microelectrodes, the "stereotrode", (McNaughton, B.L., O'Keefe, J., Barnes, C.A., J. Neurosci. Meth., 8:391-397) in which the two tips are close enough to see the same population of neurones. Unit potentials appear nearly simultaneously on both electrodes, with differing amplitudes, possibly due to the distance of the neurones from the two electrodes. Generally three to five units could be distinguished by their relative amplitudes. The tetrode is a direct extension of the stereotrode in which four wires simultaneously record the same unit potentials. It improves the resolution and increases the number of simultaneously recorded units to as many as ten. We have found several cases in which the tetrode separates units that could not be distinguished by a stereotrode. Finally, the tetrode opens up the possibility of localising the source of a unit potential in three dimensional space, allowing the study of interactions between a group of anatomically identified neurones.

490.4

AN ELECTRODE ARRAY SUITABLE FOR CHRONIC LAMINAR RECORDINGS FROM DEEP STRUCTURES IN PRIMATES. Arboleda, C.L., Halgren, E., & Drakulic, B. Sch. of Engin. & Brain Res. Inst., UCLA; Vet. Adm. Med. Ctr., Los Angeles, CA 90073.

Existing microelectrode arrays suitable for current-source density analysis are too short for chronic recordings from deep structures in primates. Safety considerations further require that the electrode be blunt-tipped and flexible, sealed, and biocompatible. Our electrodes are 42, 63, or 73 mm long. The 73 mm electrodes have 16 large (.4 mm^2) contacts at 3.5 mm intervals, and 42 small (40X40µ) contacts separated by 80µ. These are placed in a row near the electrode tip for laminar and multiunit recordings. The conducting lines are continuous with a 15 cm ribbon cable ending in an edge connector. Fabrication begins with evaporating titanium and then gold onto a flexible polyimide wafer. The metal is etched away except for the electrode pads and conducting lines. An insulating layer of polyimide is then deposited, and removed above the pads. Platinum is electroplated onto the gold, and chlorided if desired. A layer of silicone is layered between 2 wafers with the electrode pads facing out, and individual electrodes are cut using a diamond saw. The electrode has negligible cross-talk or phase-shift in vitro from .5 to 5000 Hz.
Supported by the Veterans's Administration and by USPHS (NS18741).

490.5

CONTRIBUTION OF MEDIAL SEPTUM NEURONS TO PERIODIC HIPPOCAMPAL FIELD ACTIVITY. B. H. Bland, J. J. Eggermont*, G. M. Smith*, L. V. Colom. University of Calgary, Department of Psychology, Behavioral Neuroscience Research Group, Calgary, Alberta T2N 1N4.

Cross-correlations between firings of simultaneously recorded neurons that exhibit a strong periodic pattern are difficult to interpret in terms of the underlying functional connectivity. The evaluation of the state-dependent correlations for pairs of medial septum (MS) neurons in the rat during hippocampal theta rhythm (θ) and large irregular activity (LIA) is therefore ambiguous. Under the assumption that the firing properties of MS neurons are reflected in θ and LIA, a correction procedure based on the Joint Peri Stimulus Time Histogram (JPSTH) can be applied. By interpreting θ and LIA to act as the "stimulus" for time-lagged MS activity, the JPSTH was applied to neuron pairs from the MS that were recorded simultaneously with θ and LIA. The correction for the cross-correlogram amounted from one-third to one-half of the peak value, thus substantially reducing the periodic components but not eliminating them. The procedure allowed a more reliable comparison between cross-correlations under θ and LIA states. In addition it was concluded that rhythmic hippocampal field activity is only partially accounted for by periodic activity in MS neurons.

490.6

LAMINAR GLUCOSE UTILIZATION IN THE HIPPOCAMPUS CORRELATED WITH ELECTROENCEPHALOGRAPHIC PATTERNS. G.A. Zimmerman, R.D. Vingan, S. Fox, T.H. Milhorat, L.A. Freed and D.L. Dow-Edwards. Lab of Cerebral Metabolism, Departments of Neurosurgery and Physiology, SUNY-Health Science Center, Brooklyn, NY, 11203.

The relationship between hippocampal glucose metabolic activity and electroencephalographic patterns was analyzed by cell layer in Long-Evans rats following unilateral fimbra-fornix (ff) lesioning. Rats were implanted with a unilateral hippocampal recording electrode and baseline hippocampal theta activity was measured. Animals were divided in 2 groups: Sham operation; and Unilateral f-f lesion. The lesion consisted of aspiration of the f-f and overlying neocortex ipsilateral to the recording electrode. At 1 week post-op, theta activity was recorded during treadmill walking. After 3 months, each rat was prepared for measurement of cerebral glucose metabolic activity according to the [^{14}C] 2-deoxyglucose protocol of Sokoloff, et al., 1977. Autoradiographs were superimposed on nissl sections with an Amersham imaging system and laminar glucose utilization was quantified. Analysis of hippocampi that sustained a post-lesion theta loss and those that displayed an eventual theta return demonstrates a correlation between hippocampal glucose metabolism and the presence of theta activity in hippocampal EEG. The pattern of glucose utilization only in the stratum oriens of the hippocampal CA1 region correlates with ipsilateral theta activity.

490.7

DETECTION OF AN ATROPINE-RESISTANT COMPONENT OF THE HIPPOCAMPAL THETA RHYTHM IN URETHANE ANESTHETIZED RATS. Steven E. Fox and Mark Stewart, Dept. Physiology, SUNY Health Sci. Ctr., Brooklyn, N. Y. 11203

An important pharmacological feature of the hippocampal theta rhythm in urethane anesthetized animals is its apparent sensitivity to antimuscarinic drugs. This sensitivity may be partly due to a masking of the theta frequency by increases in both higher and lower frequency EEG components that are unrelated to any residual theta rhythm. The discovery of atropine-resistant, rhythmic medial septal neurons has provided a physiological trigger for averaging EEG and unit activity after large atropine doses. Such averaging has permitted the detection of an atropine-resistant component of the hippocampal theta rhythm in urethane anesthetized rats. The post-atropine theta activity recorded from both CA1 (superficial to the pyramidal cell layer) and dentate (near the hippocampal fissure) in 15 rats was typically reduced in amplitude, but the recordings from the two locations maintained their phase relations to the septal units and to each other. The presence of this residual theta component after doses as large as 100 mg/kg indicates that it cannot be mediated by muscarinic cholinergic receptors. The coupling of the signal to the atropine-resistant septal cells strengthens our previous suggestion that these septo-hippocampal neurons are not cholinergic, and are therefore probably GABAergic.
(Supported by NIH grants NS17095 and NS07117.)

490.8

MONKEYS HAVE HIPPOCAMPAL THETA ACTIVITY.
Mark Stewart and Steven E. Fox, Dept. Physiology, SUNY Health Sci. Ctr., Brooklyn, N. Y. 11203

The hippocampal theta rhythm has been extensively studied in many sub-primate mammals. Some studies have suggested that the theta rhythm is essential for the normal behavioral functioning of the hippocampus. Efforts to generalize these implications to humans have been unconvincing since no clear examples of primate theta rhythm have been reported. Variability in the specific behavioral correlates for the theta rhythm across species suggested that monkeys anesthetized with urethane might be a more appropriate preparation for initial studies of the hippocampal EEG in primates.

Three macaques received ketamine (6-8 mg/kg im) to permit cannulation of a superficial leg vein for urethane infusion (1 g/kg iv). Two squirrel monkeys were initially ether anesthetized to permit venous cannulation. Four of the 5 monkeys (the exception being a very old squirrel monkey) showed clear rhythmic EEG activity. Coherence profiles at the theta frequency had maxima between the dentate granule cell layer and the CA1 pyramidal cell layer. Similarities with theta rhythm of urethanized rats included: 1) sensitivity of the urethane-induced theta activity to muscarinic drugs; and 2) its correlation with spontaneous movements during light anesthesia. Important differences were: 1) the frequency of this theta activity was 7-9 Hz compared to 4-5 Hz found in rats; 2) considerable amounts of low frequency EEG co-existed with the monkey theta activity; and 3) durations of bouts of theta activity in monkeys were shorter than in rats.

Primates generate hippocampal theta activity that appears to be homologous to that of rats. The similarities between the monkey and rat EEGs emphasize the utility of sub-primate preparations for developing methods for studying hippocampal EEG states in monkeys and man. (Supported by NIH grants NS17095 and NS07117.)

490.9

BEHAVIORAL CORRELATES AND CHOLINERGIC MANIPULATIONS OF HIPPOCAMPAL PHASIC LINEAR θ-ON CELLS. L. V. Colom, S. Roquet*, B. H. Bland, University of Calgary, Department of Psychology, Behavioral Neuroscience Research Group, Calgary, Alberta T2N 1N4.

The firing repertoires of phasic linear θ-on cells in the CA1 and dentate layers of the hippocampal formation of the freely moving rabbit were analyzed during 3 behavioral conditions: (1) voluntary motor patterns, termed type 1 θ behaviors; (2) automatic motor patterns, termed type 2 LIA behavior; (3) alert immobility with presentation of sensory stimuli, termed type 2 θ behaviors. Phasic linear θ-on cells discharged rhythmically during type 1 and type 2 θ behaviors and increased their discharges in a linear positive manner in relation to increases in θ frequency. These same cells discharged irregularly and at lower mean rates during type 2 LIA behaviors. The discharge rates during type 1 θ behaviors were always greater than that occurring during type 2 θ behaviors, even at equivalent θ frequencies. Administration of $ATSO_4$ abolished the rhythmicity and linearity during type 2 θ behaviors. Cellular rhythmicity and linearity during type 1 θ behaviors was not affected by the drug but mean discharge rates were reduced in all 7 cells tested ($\bar{x}$ = 28.14 ± 18.02%; range = 5-56%).

490.10

EFFECTS OF HIPPOCAMPAL LESIONS ON MACAQUES PERFORMANCE OF A TASK REQUIRING ISOMORPHIC SPATIAL MAPPINGS.
B.Q. Moore, P. Alvarez-Royo, I.Y. Polis*, H. Pashler* and G.C. Baylis. Depts. of Neuroscience and Psychology, University of California, San Diego, La Jolla, CA 92093.

The hippocampus of the primate has been strongly implicated in memory processes. Much of this research has centered around tasks such as Delayed Non Matching to Sample (DNMS). The Wisconsin General Test Apparatus necessitates that correct and incorrect responses are at different locations. Work in the rat has suggested that the hippocampus may play a central role in spatial awareness. If primates with hippocampal lesions are impaired in their representation of spatial location, this may explain a part of the deficits seen in performing tasks in which responses are spatial.

To test whether monkeys with hippocampal lesions are impaired in their representation of space *per se*, normal (n=3) and hippocampal (n=3) macaques were trained on a task in which they had to respond by pressing one of three levers directly below small squares (5 cm x 5 cm) illuminated on a video monitor in front of the monkey. A screen prevented the monkeys from seeing both the target and lever at the same time. Normal monkeys were able to perform this task after 169 (108-317) blocks of trials whereas monkeys with hippocampal lesions were unable to learn this task to criterion: training was curtailed at 500 blocks of trials. An analysis of errors showed that monkeys with hippocampal lesions were typically able to respond correctly on two out of the three locations at any time.

These results suggest that the primate hippocampus plays a role in spatial attention or awareness.

This work was supported by O.N.R. contract N00014-88-K-0281.

490.11

THE EFFECTS OF BASOLATERAL AMYGDALA AND VENTRAL STRIATAL LESIONS ON CONDITIONED PLACE PREFERENCE IN RATS. B.J. Everitt, K.A. Morris*, A.O'Brien*, L. Burns* and T.W. Robbins. Depts of Anatomy & Experimental Psychology, Cambridge Univ., Cambridge CB2 3DY, England.

The basolateral amygdala (BLA) projects richly to the ventral striatum (VS), but the behavioral functions of this "limbic-motor interface" remain unclear. In these experiments, selective, bilateral, axon-sparing lesions of the BLA and VS were made by infusing the excitotoxins, quinolinic and quisqualic acids, respectively. The effects of these lesions were investigated on the expression of (i) a conditioned place preference (CPP) acquired by prior pairings of a distinctive environment with 20% sucrose and (ii) a discriminated approach response in an operant chamber with sucrose reinforcement, in mildly deprived rats. Both BLA and VS lesions abolished the CPP, but differed in their effects on ingestive, locomotor and discriminated approach behavior. Lesions of the ventromedial, but not the dorsolateral, caudate-putamen also attenuated the CPP. Interactions between the BLA and VS were studied by unilaterally lesioning BLA together with the contralateral VS. This asymmetric lesion also markedly attenuated CPP. These results strongly implicate amygdala-ventral striatal interactions in reward-related processes and endorse the functional importance of this limbic-motor interface.

490.12

PASSIVE AVOIDANCE OF DRINKING AFTER LESIONS OF THE CENTRAL NUCLEUS OR LATERAL AREAS OF THE RAT AMYGDALA.
G.D. Coover, F.K. Jellestad*, R. Murison* and H. Ursin*, Dept. of Psychology, Northern Illinois University, DeKalb, IL 60115 and Institute for Physiological Psychology, University of Bergen, 5009 Bergen, Norway.

The effects of electrolytic or ibotenate lesions of the central nucleus (ACE), rostral lateral nucleus (AL) or caudal lateral plus basolateral nuclei (BL) of the amygdala were tested in a task where footshocks of increasing intensity were administered when the rat drank from a water spout. Passive avoidance of drinking for 5 minutes (PA) was selectively affected by electrolytic ACE lesions, where 51.8 ± 2.8 (Mean ± SEM) footshocks were taken to PA, compared to electrolytic lesions of the dorsal hippocampus, 19.7 ± 1.8 footshocks, or control surgery, 25.9 ± 2.8 footshocks. Electrolytic lesions of the rostral AL, 36.0 ± 2.0, also produced a PA deficit compared to controls, 26.4 ± 1.8, but not caudal BL lesions, 30.9 ± 4.2 footshocks.

Ibotenate (.07 μg) lesions produced a slight PA deficit when placed in ACE, 31.9 ± 1.3 footshocks, compared to controls, 23.4 ± 1.3, but not when placed in rostral AL, 26.1 ± 2.1, or caudal BL, 26.1 ± 3.1.

The results suggest that fibers passing through the central nucleus are involved in normal passive avoidance behavior, while there is some contribution from the central nucleus.

490.13

DIFFERENTIAL EFFECTS OF SOCIAL CONTEXT UPON LATERALIZATION AND AMPLITUDE OF THE ELECTRICAL ACTIVITY OF THE AMYGDALA AND CORTEX IN SQUIRREL MONKEYS. R. L. Lloyd, A. S. Kling, F. Houriani*, E. Mirzabeigi*, and O. Ricci*. Psychiatry Service UCLA/VA Medical Center, Sepulveda, CA 91343, and Biology Department, California State University, Northridge.

Electrical activity was recorded from amygdala and cortex in four animals seated in a restraining chair, alone or with a conspecific. Power spectral analysis revealed an increase in total power recorded from the amygdala when the animal was in the presence of a conspecific, reflecting increases in the a, b, and d bands but not in t. No differences were observed from any combination of cortical electrodes.

In all frequency bands, except d, power in the right amygdala exceeded left in both conditions; d in the right hemisphere exceeded that of the left only when the monkey was in isolation. Cortical a and d were greater in the left hemisphere under both conditions.

These results suggest a right bias in the processing of social/sensory information.

This study was supported by a grant from the Veterans Administration.

490.14

CATECHOLAMINERGIC DEAFFERENTATION OR CELL BODY LESIONS OF THE AMYGDALA IMPAIR FEAR CONDITIONING TO EXPLICIT BUT NOT CONTEXTUAL CUES. N.R.W. Selden*, B.J. Everitt and T.W. Robbins. (SPON: Brain Research Association). Depts of Experimental Psychology and Anatomy, University of Cambridge, Cambridge CB2 3EB, U.K.

Rats received bilateral lesions of the basolateral amygdala (BLA) by infusing either quinolinic acid (QUIN) or 6-hydroxydopamine (6-OHDA). Lesioned and sham-operated rats were water deprived and pre-exposed to a two-chambered place preference apparatus. Immediately following pre-exposure, each rat was enclosed in one chamber, where it experienced five pairings of a 30 sec, clicker CS and a 0.5 sec, 0.5 mA footshock. Half of the control group and half of each lesion group were trained with a 10 sec trace, and half with a 30 sec trace, interval between CS offset and shock onset. All rats were then re-exposed to the CS in a separate, operant chamber. Suppression of licking was used as a measure of fear conditioning to the CS, while preference for the safe side of the training apparatus measured fear conditioning to contextual cues. Consistent with the predictions of attentional theory, control rats in the 10 second trace group were more strongly conditioned to CS and less strongly conditioned to context than controls in the 30 second trace group. Both QUIN and 6-OHDA rats were severely impaired in fear conditioning to the CS, but showed normal contextual conditioning in both trace interval groups. These results suggest that catecholaminergic mechanisms in the BLA mediate conditioning to explicit, but not contextual, cues in this aversive paradigm.

490.15

INVOLVEMENT OF D1 AND D2 RECEPTOR MECHANISMS IN THE PROCESSING OF REWARD-RELATED STIMULI IN THE VENTRAL STRIATUM. G. Wolterink*, M. Cador* I. Wolterink*, T.W. Robbins and B.J. Everitt. (SPON: Brain Research Association). Depts. of Anatomy & Experimental Psychology, Cambridge Univ., Cambridge CB2 3DY, England.

Intra-accumbens D-amphetamine (AMPH) enhances the effects of reward-related stimuli, as shown using an acquisition of new response procedure with conditioned reinforcement (CR). A similar paradigm has been used here to determine the dopaminergic specificity of these effects. Thirsty rats received pairings of water and a light/noise compound stimulus (CR) prior to a test phase in the absence of water in which responses on one of two novel levers produced CR, but on the other had no effect. Intra-accumbens dopamine (5-50ug) or the D1 receptor agonist SKF38393 (0.01-10.0ug) dose-dependently and selectively increased responding on the CR lever, but the D2 receptor agonist LY171555 (0.01-10.0ug) was without effect. Noradrenaline (25-100ug) infused into the nucleus accumbens also did not affect responding for CR. The effects of intra-accumbens AMPH (18ug) were completely blocked by immediately antecedent intra-accumbens infusion of the D1 receptor antagonist SCH23390 (1ug) or the D2 receptor antagonist raclopride (5ug). These results show that both D1 and D2 receptors contribute to the effects of intra-accumbens AMPH, but that stimulation of D2 receptors in the nucleus accumbens is, by itself, insufficient to enhance the effects of CR.

490.16

HIPPOCAMPAL MODULATION OF MESOLIMBIC FUNCTION. T. L. Steele & L. D. Devenport. Department of Psychology, Univ. of Oklahoma, Norman, OK 73019.

Hungry rats with lesions of the hippocampal formation are stereotypic and hyperactive when exposed to cues associated with reward (Science, 1981, 212, 1288). These behaviors seem to be due to unchecked activation of incentive processes, possibly with a focus in the mesolimbic dopamine (DA) system. This assumption was tested behaviorally by placing food-deprived sham and hippocampal - lesion rats in an environment predictive of reward. As incentive motivation was intensified by increasing deprivation, sham rats became hyperactive and engaged in stereotypic behaviors identical to those displayed by hippocampal rats. Stereotypy, however, did not exceed levels commonly associated with mesolimbic DA activation in any of the animals. Based on these results, free-fed rats in a second study were given one of five doses of d-amphetamine (0-3 mg/kg) to determine if the lesions would selectively enhance low dose stereotypies while leaving high dose stereotypies unaffected. These expectations were confirmed. It is apparent that the hippocampus influences the mesolimbic system, while having little effect on behaviors associated with nigro-striatal activity.

490.17

DISTINCT TH AND DBH IMMUNOREACTIVE FIBERS IN THE MONKEY'S HIPPOCAMPUS. Y.Samson*, J.Wu*, A.Friedman*, M. Deschuyteneer*, J.N.Davis. (SPON: J. HALL) V.A. Medical Center and Duke University Medical Center, Durham, NC 27710.

The distribution of TH and DBH immuno-reactive fibers was studied in the hippocampal formation (dentate, CA3, CA1, and subiculum) of Cynomolgus monkeys. Four hippocampal formations from two normal monkeys, and two from one monkey eleven days after bilateral fornix lesion were studied. In normal monkeys, the density of TH fibers was very high in the hilus and the molecular layer of the dentate gyrus; high in the 3 layers of the subiculum, and in stratum lacunosum-moleculare of CA1 and CA3. Only rare TH fibers were found in the remaining layers of CA1 and CA3, and in stratum granulosum of the dentate gyrus. By contrast, the density of DBH fibers was moderate or low, but DBH fibers were present in all areas except stratum granulosum of the dentate. In the animal studied after fornicotomy, a substantial decrease in both TH and DBH immunoreactive fibers was limited to the rostral hippocampus. Since TH antiserum may preferentially label dopaminergic fibers, while DBH antiserum may label noradrenergic fibers, these findings suggest a moderate but widespread noradrenergic innervation of the monkey's hippocampus, and a heavier, more restricted dopaminergic innervation. The loss of fibers after fornix lesion only in rostral hippocampus suggests that, as in rodents, these catecholamine fibers may reach the hippocampus through at least two distinct paths.

(Supported by the NIH (NS06233) and the VA).

490.18

ASSESSMENT OF DAMAGE FROM IMPLANTATION OF MICRODIALYSIS IMPLANT IN THE HIPPOCAMPUS: A. Shuaib*,A.-L. Siren, K. Xu, G. Feuerstein, B.G. Crain, G. Miller*, J.N. Hallenbeck*, J.N. Davis. VA, Duke and Bethesda Naval Centers, Duke University and USUHS, Durham N.C., Bethesda M.D.

We used silver degeneration staining (SDS) to assess the extent of neuronal damage in the rat hippocampus after insertion of dialysis probes. Probes were stereotaxically placed in the hippocampus in Sprague-Dawley rats and the animals were sacrificed at varying intervals. Neuronal degeneration was evident in all animals. Cell body argyrophilia was usually restricted to a 2 to 3 cell layer immediately adjacent to the probe pathway. In addition, there was wide spread argyrophilia of well defined anatomical pathways. Damage was present 1)in the perforant path in CA1 and the dentate both proximal and distal to the implant,2) in the associational fibers in the stratum radiatum and stratum oriens in CA1 and CA3 adjacent to the probe and 3) occasionally in the commisural fibers of the contralateral CA1 region. When the probe penetrated regio inferior, damage was present in CA3 mossy fibers and in Schaffer collaterals in both CA3 and CA1.

While some damage is invariable when an object is implanted in the brain, both local damage and damage to fibres of passage should be taken into consideration when the results of in-vivo dialysis are analyzed. (Sponsored by VA and DOD)

INTERACTIONS BETWEEN NEUROTRANSMITTERS IV

491.1

SYSTEMIC KAINIC ACID INCREASES TRH PROHORMONE mRNA EXPRESSION IN RAT CNS. M.S. Kreider, M.E. Lewis, F. Baldino, Jr. and A. Winokur. Dept. of Psychiatry, U. of PA, Phila., PA 19104 and Cephalon, Inc., West Chester, PA 19380

Previous studies in our laboratory have shown that induction of limbic seizures by systemic administration of kainic acid (KA) produced large increases in the concentration of TRH in limbic regions of the rat CNS (Kreider et al, Neurosci Abs. 13:1656, 1986). We investigated whether systemic KA administration produces an increase in the expression of TRH prohormone mRNA prior to the observed increases in TRH concentration.

Male Sprague-Dawley rats (180-200g) received s.c. injections of KA (12 mg/kg in 0.9% NaCl) or vehicle. All rats were sacrificed 6 hours later and their brains rapidly frozen. 30um sections were cut and affixed to gelatin subbed slides. In situ hybridization studies were conducted using a [35S]dATP labeled synthetic oligonucleotide probe complementary to TRH prohormone mRNA.

KA administration resulted in significant increases in TRH prohormone mRNA expression were observed in the CA2 and CA3/CA4 pyramidal cell layers of the dorsal hippocampus and the basomedial, central, and medial amygdaloid nuclei. In addition, increases were observed in the dorsal and ventral endopiriform nuclei and in the perirhinal and piriform cortices. TRH prohormone mRNA expression in hypothalamic nuclei was not altered following KA administration. These results indicate that the KA-induced regional increases in TRH concentrations are preceded by an increase in TRH prohormone mRNA expression.

491.2

NICOTINE INTERFERES WITH INHIBITORY PROCESSES IN MOUSE HIPPOCAMPUS. R. K. Freund, V. Luntz-Leybman* and A. C. Collins. Instit. for Behavioral Genetics, Univ. of Colorado, Boulder, CO 80309

The excitatory effects of nicotine (Nic) in the hippocampus may be due to interference of GABA transmission [Freund et.al. Brain Res. 453 (1988) 215]. Here, paired-pulse experiments were used to learn whether Nic affects endogenous inhibitory systems in hippocampal slices from DBA/2J mice. Pairs of pulses were delivered to CA1 pyramidal cells at short interstimulus intervals (5-200 ms), while recording CA1 population spikes (PS). In one series of experiments (OO), both pulses were delivered orthodromically (O) to the Schaffer collateral fibers, such that both feed-forward and recurrent inhibitory fibers are activated by the conditioning pulse. In another series (AO), the O pulse was conditioned by a preceding antidromic (A) pulse to the alveus; this paradigm should activate only recurrent circuits. Normally, an A-conditioned PS is inhibited relative to the unconditioned response. For the AO experiments, Nic relieved this inhibition, or facilitated the conditioned PS, in a concentration-dependent manner (50-400 uM). For the OO experiments, Nic (200 uM) inhibited the conditioned PS. Nic may inhibit GABA transmission in either case, but the consequences of impaired GABA transmission depend on which fibers were used for conditioning.

(Supported by R. J. Reynolds Tobacco Co. and DA-00116)

491.3

NMDA RECEPTOR ANTAGONISTS IMPAIR RETENTION IN RATS AND POTENTIATE 5HT-STIMULATED PI HYDROLYSIS IN HIPPOCAMPAL SLICES
O.Gandolfi, P.Roncada* and R.Dall'Olio*. Institute of Pharmacology, University of Bologna, Italy.

APV (5 µg/10 µl i.c.v.) or ketamine (12.5 mg/kg i.p.), administered 5 min before the training session of a passive avoidance conditioning, failed to changes step-through latencies when compared to saline-treated rats; in contrast both drugs decreased the latency on test session (24 hrs later). Since our previous data suggest a role of serotonin (5HT) in memory retention, it was intriguing enough to study whether 5HT-induced phosphoinositide (PI) hydrolysis could be affected by the administration of NMDA antagonists. 5HT-induced PI hydrolysis was evaluated by prelabeling hippocampal or cortical slices with ^{3}H-myo-inositol and by measuring the accumulation of ^{3}H-inositol monophosphate in the presence of lithium ion. In hippocampal or cortical slices of APV and ketamine treated rats, 5HT-stimulated PI metabolism was potentiated in comparison to controls. These results suggest that EAA, besides their role as neurotransmitters, could also interact with other mediators in the production of second messengers in rat brain. What physiological role of EAA-5HT interactions could play in memory retention opens questions for further investigations.

491.4

GLUCOCORTICOIDS SUPPRESS EXCITABILITY IN HIPPOCAMPUS.
M. Joëls* and E.R. de Kloet. Div. of Molecular Neurobiology, Inst. of Molecular Biology and Medical Biotechnology, and Rudolf Magnus Inst., University of Utrecht, Utrecht, The Netherlands.

Pyramidal cells in the CA1 region of the rat hippocampus are a major target for adrenal corticosteroids. With intracellular recording we studied the effects of glucocorticoids on CA1 cells in hippocampal slices. The steroids were perfused for 20 minutes, followed by a 30 to 90 minutes period of washout prior to recording. Such prior perfusion of slices from adrenalectomized (ADX) rats with 1 µM corticosterone or 1 nM-1 µM of the glucocorticoid agonist RU28362 markedly enhanced both the amplitude and duration of the afterhyperpolarization (AHP) induced in CA1 neurons by a 0.5 nA cathodal pulse (50 or 500 ms duration). The steroid effects on AHP were blocked by the glucocorticoid antagonist RU38486. The action of the steroids is probably mediated by a postsynaptic mechanism since AHPs were still affected by the steroid while TTX (1 µM) and TEA (5 mM) were present. Preliminary observations indicate that the steroid prolonged the calcium-spike recorded under these conditions. We also found that the AHP in slices from ADX rats was significantly smaller than the AHP in slices from sham-controls, although no differences existed in overall resting membrane properties. While steroid hormones thus enhance the AHP, activation of the β-receptor by norepinephrine (NE) has been reported to diminish the AHP and spike accommodation. The latter effects of NE were more pronounced in slices from ADX rats than either in slices treated with steroids or in slices from sham-controls. Our data suggest that selective activation of the glucocorticoid receptor in CA1 cells enhances the AHP, possibly by a genomic action on the calcium metabolism. Both the direct effect of the glucocorticoids on the AHP and the effect on the NE-induced blockade of cell accommodation will result in a steroid-induced decrease of neuronal excitability in the hippocampus.

491.5

THE EFFECTS OF CARBACHOL ON EXTRACELLULARLY RECORDED RESPONSES IN THE RODENT DENTATE GYRUS MAINTAINED *IN VITRO*.
J.S. Kahle and C.W. Cotman, Dept. of Psychobiology, University of California, Irvine, CA 92717.

Dentate gyrus granule cells receive excitatory amino acid-using afferents from entorhinal cortex (perforant path) which terminate in the outer two-thirds of the molecular layer. This same region receives cholinergic input from the medial septum suggesting the possible interaction between these transmitter systems in the dentate gyrus. Indeed, applications of carbachol (a general acetylcholine agonist) reduce synaptic responses of dentate granule cells evoked by perforant path stimulation. Recently, we investigated the effects of carbachol on extracellular field potentials (EFPs) recorded from rodent hippocampal slices maintained *in vitro*. Applications of carbachol reduced EFPs recorded from the middle third of the molecular layer, the terminal region of the medial perforant path (MPP), whereas responses recorded from the outer third of the molecular layer (lateral perforant path EFP) were not reduced.

Pharmacological studies indicate that carbachol-induced reduction of MPP EFPs is mediated by muscarinic acetylcholine receptors as indicated by atropine (a muscarinic specific antagonist) sensitivity. More detailed pharmacological studies suggest that this effect may be mediated specifically by the M_2 muscarinic receptor subtype in the dentate. Co-applications of carbachol (1µM) and gallamine (an M_2 preferring antagonist; 20µM) protect against carbachol-induced EFP reduction (75%), whereas co-applications of carbachol (1µM) and pirenzepine (an M_1 preferring antagonist; 100nM) do not protect against this effect.

These results may be explained by the presence of a presynaptic inhibitory action of carbachol mediated by cholinergic M_2 receptors localized in the MPP terminal region.

491.6

DETERMINATION OF A TIME-RESPONSE CURVE FOR ACUTELY INTUBATED ASPARTAME IN FISCHER 344 AND SPRAGUE-DAWLEY RATS.
G.B. Freeman, T. Sobotka[a], D. Hattan[a]* Battelle Columbus Laboratories, Columbus, Ohio 43201 and [a]FDA, Washington, D.C., 20204

This study was the first in a series to define a rodent model to document the effects of amino acid-modulating compounds on central neurotransmitter function. A time response curve for a single oral dose of aspartame was determined in unfasted male Fischer and Sprague-Dawley rats. Regional brain concentrations of NE, DA, 5-HT and their metabolites were analyzed in the hypothalamus, cerebellum, pons/medulla, hippocampus, striatum, cortex and midbrain/thalamus at 30, 60, 120 or 240 minutes after oral aspartame (1000 mg/kg) administration. The acute administration of aspartame had little effect on static levels of the catecholamines or indoleamines. No one strain had a greater or different sensitivity to aspartame than the other. Although the present study indicated that aspartame was without effect on monoamine metabolism, these data should be interpreted cautiously in view of the fact that data on blood and brain levels of the amino acids, phenylalanine and tyrosine, have not been determined as well as direct measurements of synthesis or turnover. In addition, the present study used unfasted rats which may have increased the possibility of aspartame binding to feed in the stomach. Several of these issues are being addressed in an experimental study currently in progress.

491.7

SEROTONIN (5-HT) DEPLETION UNMASKS 5-HT COMPONENT OF [^{3}H] DIHYDROALPRENOLOL ([^{3}H]DHA) BINDING IN RAT BRAIN.
C.A. Stockmeier and K.J. Kellar. Dept. Psychiatry, Case Western Reserve Univ., Cleveland, OH 44106; Dept. Pharmacology, Georgetown Univ., Washington, DC 20007.

Lesions of 5-HT neurons lead to an increase in [^{3}H]DHA binding sites in rat brain. Previously, we interpreted this increase in binding as an increase in beta-adrenergic receptors. Despite our earlier report, extensive studies show that the increase in binding is not accompanied by an increase in agonist-stimulated accumulation of cyclic AMP. Lesions of 5-HT neurons with p-chloroamphetamine (PCA) increase [^{3}H]DHA binding which is blocked in vitro by 5-HT, metergoline, RU-24969, or TFMPP (EC_{50}=1-3 nM), but not by 8-OH-DPAT, spiperone, mesulergine, dopamine, or norepinephrine. [^{3}H]8-OH-DPAT or [^{125}I]cyanopindolol binding to 5-HT-1A or 5-HT-1B receptors, respectively, was not increased by PCA despite a marked increase in [^{3}H]DHA binding in the same tissues. However, when tissues from controls or PCA-treated rats were preincubated at 37°C for 10' to remove endogenous 5-HT bound to receptors, the binding of [^{3}H]DHA in controls was increased to the level seen in PCA-lesioned tissues. Thus, [^{3}H]DHA binds primarily to beta-adrenergic receptors in control membranes which are not preincubated. Either preincubation of control tissues, or 5-HT depletion by PCA, unmasks a 5-HT-1 receptor subtype to which [^{3}H]DHA binds in addition to the beta-adrenergic receptor. Supported by NS24523, MH41819, and MH41684.

491.8

EFFECT OF SOCIAL CONFLICT ON BRAIN DOPAMINE IN MICE: INTERACTIONS WITH NALTREXONE.
A.Malnoe* [1], K.Ornstein [1] and B.Siegfried* [2].
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It has been shown that social conflict activates brain opioid systems in mice (Külling et al, Brain Research 450:237-246, 1988). Moreover, opioids are known to modulate dopamine (DA) neurotransmission within the nigrostriatal and mesolimbic systems. In the present study, we have investigated the effect of social conflict on brain DA metabolism in DBA/2 mice as well as the role of endogenous opioids in stress-induced changes in dopaminergic activity. Mice were pretreated with either saline or the opiate antagonist naltrexone (2 mg/kg i.p). Ten minutes later, mice were confronted either to a nonaggressive or to an aggressive opponent of the ICR strain. The aggressive confrontation was terminated after 30 bites. Attacked mice were sacrificed 20 minutes later and the levels of DA, DOPAC and HVA in different brain regions were determined by HPLC with electrochemical detection. Aggressive confrontation increased the levels of DOPAC in hypothalamus, frontal cortex, and periaqueductal gray but had no effect in the striatum, olfactory bulb and olfactory tubercle/nucleus accumbens. The increases in DOPAC were unaffected by naltrexone. In contrast, naltrexone blocked the increase in DA levels induced by aggressive confrontation in the frontal cortex and periaqueductal gray of DBA/2 mice. These results suggest that endogenous opioids mediate the social conflict-induced increases in DA synthesis in these brain regions.

491.9

CHRONIC YOHIMBINE TREATMENT GIVEN TO MORPHINE-DEPENDENT RATS ATTENUATES NALOXONE-PRECIPITATED WITHDRAWAL WITHOUT ATTENUATING ANALGESIA. J.R. Taylor, J.D. Elsworth, V.Q. Lewis*, R.H. Roth and D.E. Redmond, Jr. Depts. of Psychiatry and Pharmacology, Yale Univ. Sch. of Med., New Haven, CT. 06510.

Noradrenergic neuronal hyperactivity after chronic morphine administration has been suggested to be partially responsible for the signs and symptoms of opioid withdrawal. If the suppression of noradrenergic activity that occurs during morphine administration could be prevented, this might also prevent withdrawal effects. To test this hypothesis, we gave the alpha-2-antagonist yohimbine to rats, in order to increase noradrenergic activity, during morphine treatment. Withdrawal was subsequently precipitated with naloxone. There were six groups; saline controls (N=11), morphine (N=11), morphine + 2.0 or 3.0 mg/kg/day yohimbine (N=15 or N=5), 2.0 or 3.0 mg/kg/day yohimbine (N=11 or N=5). Subjects received 75-mg morphine pellets or sham-pelleting, on day 1, 4 and 6 of the treatment. Yohimbine was delivered throughout the morphine treatment by s.c. implanted osmotic pumps. On the seventh day, all subjects were given 1.0 mg/kg naloxone and rated for behavioral symptoms of withdrawal. Analgesia was also measured, by observing tail flick latencies (TFL) before treatment and before the test. Naloxone precipitated withdrawal only in those subjects that received morphine alone. Withdrawal was attenuated by the concurrent administration of yohimbine and morphine: Wet-dog shakes, teeth chattering, irritability, rhinorrhea and abnormal posture were significantly reduced compared with subjects treated with morphine alone. Although concurrent morphine and yohimbine treatment attenuated naloxone-precipitated withdrawal from morphine, analgesia measured by TFL was not attenuated. It is suggested that yohimbine-induced activation of alpha-2 adrenergic receptors blocks withdrawal without reducing therapeutically useful opioid analgesia.

Support by USPHS MH31176, DER by RSA MH00643

491.10

DIFFERENTIAL CROSSTALK BETWEEN GLUTAMATE AND MUSCARINIC CHOLINERGIC RECEPTORS R.Levy* and R.Simantov (SPON:Y.Shavit) Dep. of Genetics, Weizmann Ins. of Sci., Rehovot, Israel

Fetal brain cell cultures and several neuronal cell lines that bind the muscarinic receptor ligand ^{3}H-QNB were used to investigate whether excitatory amino acids, particularly glutamate, modulate the expression of muscarinic receptors. This was based on the finding that chronic membrane depolarization with KCl has a selective regulatory effect on the binding of ^{3}H-QNB to cells expressing different receptor subtypes (Cell Mol. Neurobiology, 9, 87-94, 1989), and the observation that the Na^+ channel activator veratridine has a selective effect on ^{3}H-QNB binding to neuronal cultures of different brain regions like the septum and hippocampus. Thus, muscarinic receptors in different brain regions may respond to chronic activation, by exogenous or endogenous inducers, according to the receptor's pre- or postsynaptic function. Glutamate induces time- and dose-dependent effect on the binding of ^{3}H-QNB to the M4 receptor subtype of the NG108-15 cells, with a Scatchard analysis indicating an increased affinity of the ligand to the receptor. However, the same treatment has no such effect on ^{3}H-QNB binding to cells expressing M2 muscarinic receptors. Glutamate selectivity was further indicated by that the amino acid has no effect on δ type opioid receptors of the NG108-15 cells. The intracellular pathways that may be involved in this selective crosstalk between glutamate and M4 muscarinic receptors is discussed.

491.11

INTERACTION BETWEEN TRACE AMINES WITH BIOGENIC AMINES REFLECTED IN SYNAPTOSOMAL MEMBRANE CHANGES. J. Harris, S. Trivedi* and B.L. Ramakrishna*. Chemistry Dept., Arizona State University, Tempe, AZ 85287-1604

The question regarding a neuromodulatory role of "trace" amine neurotransmitters was explored using spin labelled lipid markers to follow changes in the organizational state (membrane fluidity) of isolated synaptosomes. Initial observations revealed that the membrane fluidity induced by phenylethylamine (PE) or dopamine (DA) respectively, became highly disorganized with PE and DA with its agonist apomorphine (APo), which increased membrane disorganization more intensively than DA. In the presence of PE and and Apo, in contrast to PE and DA, the fluidity of the membrane remained unaltered from -28°C to 0°C but decreased the Apo effect on fluidity in the temperature range 5°C to 37°C. The addition of MAO inhibitor nialamide (Nial) to PE and DA did not affect the increased fluidity induced by PE and DA. In experiments using benzylamine (BZ) for PE, a greater increase in fluidity was induced than that obtained from PE. Nial did not change the BZ effect on fluidity but did alter the BZ and DA effect. These results cannot be attributed to MAO activity. In addition, Apo has effects on the synaptosomal membrane beyond that on DA receptor sites. Experiments with the interaction of tyramine (TA) and norepinephrine (NE) and with tryptamine (TRY) and serotonin (5HT), provided support for a neuromodulation role of trace amines of biogenic amine neurotransmitters.

491.12

EFFECTS OF ORGANOPHOSPHATE INTOXICATION ON LEVELS OF CENTRAL TRANSMITTERS AND THEIR METABOLITES MEASURED WITH HPLC. M. El-Etri*, W.T. Nickell, and M.T. Shipley. Dept. of Anatomy and Cell Biology, University of Cincinnati College of Medicine, Cincinnati, Ohio 45267.

Muscarinic receptors are known to modulate the release and possibly the metabolism of other central transmitters; thus, the symptoms of AChE inhibition by organophosphate poisoning may result partially from secondary effects of excess ACh on these other transmitters. We have measured changes in the levels of biogenic amines in the olfactory bulb and striatum produced by sub-lethal doses of the irreversible cholinesterase inhibitor, soman.

Adult male Sprague-Dawley rats were injected i.m. with 0.7xLD50 of soman in saline; control rats were injected with an equal volume of vehicle. Twenty-four hours following the injection, surviving animals were killed by decapitation. The brains were rapidly removed, the olfactory bulbs and striata were dissected, and the samples were quickly frozen on dry ice. The levels of 3,4-dihydroxyacetic acid (DOPAC), homovanillic acid (HVA), dopamine, norepinephrine, 5-hydroxyindoleacetic acid (5-HIAA), and serotonin were determined by HPLC/EC.

The most significant changes produced by soman intoxication were an increase in 5HIAA, the major metabolite of serotonin, in the striatum, and a decrease in DOPAC and HVA, the major metabolites of dopamine, in both structures. These results suggest that excess ACh produced by AChE inhibition alters the metabolism and release of dopamine and serotonin in two forebrain structures. Thus, therapeutic approaches to organophosphate poisoning might be improved by treatment of these secondary effects. (Supported by DAMD 17-86-C-6005 and NS23348).

491.13

THE EFFECTS OF AMPHETAMINE AND PILOCARPINE ON THE RELEASE OF ASCORBIC AND URIC ACID IN SEVERAL RAT BRAIN AREAS. K. Mueller and P.M. Kunko. Dept. of Psychology, Texas Christian Univ., Fort Worth, TX 76129

Ascorbic acid (AA) is present in high concentrations in brain. Because extracellular AA varies in response to psychoactive drugs, AA may have some function in brain. However, most research on AA has been limited to striatum and to dopaminergic drugs. Even fewer data are available on uric acid (UA); extracellular UA also varies in response to psychoactive drugs.

Linear sweep voltammetry was used to investigate the effects of amphetamine (which enhances the release of dopamine) and/or pilocarpine (a cholinergic agonist) on the release of AA and UA in brain areas differing in dopamine and acetylcholine concentrations. In caudate, nucleus accumbens, and hippocampus, the magnitude of the amphetamine-induced increase in AA was roughly correlated with dopamine content of the brain area tested. Cingulate cortex was a notable exception. Pilocarpine produced the greatest increase in AA in cingulate cortex, even though cingulate cortex has the lowest acetylcholine concentration of the brain areas tested. The AA data were consistent with the hypothesis that amphetamine and pilocarpine release different pools of AA. The UA data were consistent with the hypothesis that amphetamine and pilocarpine release the same pool of UA.

491.14

DEVELOPMENT OF A NEUROBIOLOGICAL ANALYZER FOR THE SIMULTANEOUS DETERMINATION OF MULTIPLE COMPONENTS IN TISSUE, CSF, BLOOD AND URINE SAMPLES USING LIQUID CHROMATOGRAPHY WITH MULTIPLE, AMPEROMETRIC ELECTROCHEMICAL DETECTION. D.J. TURK* AND C.L. BLANK, (SPON: H.D. Christensen), Dept. of Chemistry and Biochemistry, U. of Oklahoma, Norman, OK 73019.

The separation and determination of a variety of catecholamine, indoleamine and acetylcholine related neurochemicals are performed using a Neurobiological Analyzer (NEUBA). The device consists of four individual liquid chromatographic systems, one autoinjector, one multiple potential-controlling/current-monitoring potentiostat, and is interfaced to a computer for data handling and reduction. Three of the LC systems each contain an electrochemical cell with 4 glassy carbon electrodes in a series arrangement. This provides a three dimensional picture for the separation and determination of the electroactive components of interest. The fourth system utilizes a single platinum electrode and is devoted to the determination of the acetylcholine related components. The multiple LC systems offer a substantial reduction in the time required for the analysis of each sample. A sample is loaded into the autoinjector and subsequently routed to each of the four systems in a rapid sequence such that all systems can be simultaneously monitored. The three primary LC systems have been optimized for the resolution of selected neurotransmitters, modulators (e.g., norepinephrine, dopamine, serotonin, melatonin, epinephrine) and related metabolites. These substances may be conveniently divided into rapidly, moderately, and strongly retained components from a chromatographic standpoint. At the present time the device is capable of identifying and quantitating approximately 20 such species using simple retention time and peak height data. However, we expect to increase the number of accessible components to 150-200 in the near future by incorporating the wealth of electrochemical information provided by the multiple detectors. The device was employed with mouse brain tissue samples to demonstrate its utility.

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SYMPOSIUM. MELATONIN: NEW LIGHT ON CNS MECHANISMS OF ACTION. D.N. Krause, Univ. of California Col. Med., Irvine and M.L. Dubocovich, Northwestern Univ. Med. Sch. (Chairpersons); J.S. Takahashi, Northwestern Univ.; S.M. Reppert, Massachusetts Gen. Hosp.; V.M. Cassone, Texas A & M Univ.

Recent key studies have shed new light on the mechanisms by which melatonin affects CNS function to regulate a variety of processes including biological rhythms, neuroendocrine systems, sleep and retinal physiology. Dr. Takahashi will address the origins of CNS melatonin and *in vitro* studies on the cellular mechanisms controlling the circadian secretion of melatonin from the pineal and the retina. Dr. Dubocovich will discuss the identification and pharmacological characterization of neural receptors for melatonin using quantitative, *in vitro* functional and radioligand binding assays and the development of selective receptor agents, including the first competitive antagonist luzindole. Dr. Reppert will present autoradiographic studies which visualize melatonin receptors in discrete CNS regions such as the suprachiasmatic nucleus (SCN), site of a putative biological clock, and the median eminence in adult and fetal brains from rodents and humans. Dr. Cassone will discuss data demonstrating that melatonin acts via the SCN to entrain circadian rhythms in a number of species including man and will comment on the clinical implications of melatonin's entraining effect for treating circadian rhythm disorders such as jet lag, sleep disorders and depression.

VISUAL PSYCHOPHYSICS AND BEHAVIOR III

497.1

COLOR FILLING: PSYCHOPHYSICAL EVIDENCE AND A NEURAL NETWORK MODEL. M.A. Paradiso and K. Nakayama, Smith-Kettlewell Eye Research Institute, San Francisco, CA 94115

If a person with a retinal scotoma has their scotoma surrounded by a visual target of a single color, that color will appear to fill-in the blind spot. Experiments with images stabilized on the retina of normal subjects demonstrate that in this special situation filling-in can also occur in people with intact visual systems. We will report the results of experiments which strongly suggest that even in normal (non-stabilized) vision there is a dynamic temporal filling mechanism underlying the perception of homogeneously colored regions. In one of our experiments a large disk-shaped target of a single color was briefly presented to an observer and shortly thereafter a white circular mask of smaller radius was presented. The mask was found to have a strong suppressive effect on the perceived brightness of the center of the disk. Experiments in which the radii of the target and mask were varied suggest that color propagates from the edges of the homogeneously colored target. The mask appears to interrupt or interfere with this filling process. We have examined several different neural network models, some of which have been used by others to account for color vision, to determine whether they can account for our findings. The models have the common feature that color borders excite cells and this activation spreads across the network because of lateral excitatory connections. The models differ in the manner in which the color is bounded. For instance, lateral inhibition or shunting inhibition can effectively stop the spread of a color into inappropriate areas. We will present a shunting model which has been successful in accounting for our experimental results.

497.2

ILLUSORY CONTOUR ORIENTATION DISCRIMINATION IN THE CAT. P. De Weerd*, E. Vandenbussche* and G.A. Orban (Spon: B. Gulyas). Lab. Neuro- en Psychofysiologie, K.U.Leuven, Campus Gasthuisberg, B-3000 Leuven, Belgium.

We present the first evidence that cats are able to discriminate fine orientation differences of illusory contours. Two types of illusory contour have been used (after Vogels and Orban, Vision Res., 27:453, 1987). For both illusory contour types, 30 daily sessions sufficed to train each of 2 cats to threshold for 2 reference orientations (horizontal and right oblique). This rapid teaching was achieved by using a training method which reduces exposure to the negative stimulus as long as threshold level is not reached (De Weerd et al., Behav. Brain Res., in press). Thresholds were measured using a 73.5% correct Wetherill and Levitt staircase as well as the method of constant stimuli and were identical for both reference orientations. The magnitude of the thresholds was lowest (10-15 degrees) if 3 or 4 pairs of inducing circle halfs constituted the illusory contour. Both increasing or decreasing this number deteriorated discrimination performance. When only the outer pair of inducing circle halfs was present, no reliable thresholds could be measured, suggesting that local cues are insufficient for the cat to solve the discrimination. This was confirmed in a control experiment, showing increasing thresholds as a function of random rotation over increasing angles of all pairs of inducing circle halfs. As in humans, orientation discrimination thresholds obtained with illusory contours are elevated compared to those obtained using real lines (less than a factor 2 in humans, a factor 2-3 in cats).

497.3

EFFECT OF HIGHER CORTICAL ABLATIONS ON CAT ORIENTATION DISCRIMINATION. E. Vandenbussche*, J.M. Sprague, P. De Weerd* and G.A. Orban (Spon: J. Duysens). Lab. Neuro- en Psychofysiologie, K.U.Leuven, Campus Gasthuisberg, B-3000 Leuven, Belgium, and Dept. of Anatomy, Sch. of Med., Univ. of Pennsylvania, PA 19104-6058.

It is established that either areas 17 or 18 are required for cats to achieve fine orientation discriminations (Sprague et al., Soc. Neurosci. Abstr., 13:1450, 1987). The present study was undertaken to test whether further cortical processing is required as predicted by most models (e.g. Vogels and Orban, Vision Res., 27:453, 1987). Areas 17 and 18 project chiefly to areas 19, 20, 21 and the medial bank of the lateral suprasylvian sulcus (Symonds and Rosenquist, J. comp. Neurol., 229:1, 1984). We ablated these areas in two cats trained to make fine orientation discriminations at two reference orientations (preoperative thresholds 4-5°). In one cat this ablation had minimal effect, even at very low contrasts (log $\Delta I/I$ = -.5). In the other, the threshold was raised by about 2°, but was probably due to the undercutting of 17 and 18, found in the histological controls. Thus ablation of these areas had little effect when made in well-trained cats, it had however dramatic effects in naive animals. In these animals even at high contrast and long line length, thresholds increased by a factor 3. We conclude that these areas are not required for further computation of orientation differences once the animal is well-trained, but are involved in acquisition of the task. Whether the latter effect is due to interference with visual processing itself or with some other factor remains to be elucidated. (supported in part by Res. Foundation, U. of Penn. to JMS)

497.4

LESIONS OF AREA 18 IN THE CAT REDUCE SENSITIVITY TO DRIFTING, LOW SPATIAL FREQUENCY TARGETS. Tatiana Pasternak, Marc Dorfman*, and John H.R. Maunsell, *Depts of Neurobiology and Anatomy, Physiology, and Center for Visual Science, University of Rochester, Rochester, NY 14627*

Psychophysical studies in cats and humans show that stimulus motion is discriminated best when the targets move briskly and are of low spatial frequency. Such targets provide optimal stimulation for neurons in cortical area 18 of the cat. To assess the contribution of this area to motion processing, we placed unilateral ibotenic acid lesions in physiologically identified portions of area 18 in two cats. The lesions were centered in the representation of the lower right visual field, about 9 deg from the vertical meridian. We measured detectability of various spatiotemporal targets placed within the ablated and intact portions of the visual field representations, while monitoring eye position with a scleral search coil. The cats were required to maintain fixation on a laser spot and respond to the presence or the absence of a grating by pressing a right or left pedal. We found a nearly 10-fold loss of sensitivity to low spatial frequency (0.3 c/deg) gratings drifting at 4.5 Hz, placed within the ablated representation of the visual field. The sensitivity loss decreased at higher spatial frequencies and the resolution limit measured in this part of the visual field was identical to that measured in the intact hemifield. Since sensitivity for discriminating stimulus direction is maximal for low spatial frequency gratings, drifting at higher speeds, the inability to detect such targets after lesions of area 18 suggests that this cortical area represents an important stage in cortical processing of motion signals. (Supported by EY06175, EY01319)

497.5

ROLE OF THE MAGNOCELLULAR PATHWAY IN PRIMATE VISION *W. H. Merigan, C. E. Byrne and J. Maunsell,* Depts. Ophthalmology, Neurobiol and Anat. and Physiol., Univ. Roch. Med. Ctr. Rochester, N.Y. 14642.

The magnocellular retino-geniculate pathway comprises only about 10% of retinal ganglion cells, but it provides the major input to important cortical structures. Its cells have large dendritic fields and large axons, and physiologically they show broad-band response to wavelength, high contrast sensitivity, and good resolution for high temporal frequencies. In this study, we examined the role of this pathway in vision by making ibotenic acid injections in magnocellular layers of the lateral geniculate, and then testing psychophysical thresholds in portions of the visual field corresponding to the resulting lesions.

Lesions at 6 deg eccentricity along the temporal horizontal meridian did not reduce contrast sensitivity for the detection of moderate spatial frequency (2 c/deg) gratings presented either without modulation, or with 10 Hz counterphase modulation. However, such lesions did reduce flicker resolution, and the magnitude of this effect was greater at lower contrasts. The stimulus in the latter experiment contained higher temporal and lower spatial frequencies than the 2 c/deg - 10 Hz stimulus for which no loss was found. This result suggests that contrast thresholds may be mediated by the magnocellular pathway at either high temporal or low spatial frequencies. This implication, as well as the role of this pathway in visual discriminations, are now under study.

Supported by grants AFOSR , ES01247, and EY01319.

497.6

EFFECTS OF INFEROTEMPORAL CORTEX LESIONS ON PATTERN-FROM-MOTION DISCRIMINATION IN MONKEYS. K.H.Britten*, W.T. Newsome, and R.C. Saunders. Stanford University and NIMH.

It is known that removal of inferotemporal (IT) cortex causes permanent pattern discrimination deficits in monkeys when the patterns are defined by luminance cues. Such deficits are observed in retention of preoperatively learned discriminations and in the rate of acquisition of new discriminations. It is not known whether such deficits obtain when the patterns are defined solely by relative motion cues. This question is of interest since visual cortical areas that are specialized for the analysis of motion project chiefly to parietal cortex, and less to temporal cortex. This issue was addressed by comparing the performance of monkeys with IT lesions against that of paired controls on 2D pattern-from-motion discrimination problems. The pattern-from-motion discriminanda consisted of high-density, high-contrast dynamic random dots presented within 2 circular apertures on a CRT screen. Each aperture contained a simple geometrical figure within which the dots moved antiparallel to those in the surround. The monkeys received a liquid reward for selecting the "positive" pattern in each pair. The monkey indicated its choice by fixating one aperture for 2 seconds. After acheiving criterion performance on 2 pattern-from-motion and 2 pattern-from-luminance problems, 3 monkeys received bilateral ablation of areas TE and TEO. As expected, the animals with IT lesions had severe deficits of both retention and acquisition of luminance-based discriminations. For pattern-from-motion discriminations, retention of preoperatively learned problems was impaired, but postoperative acquisition was unaffected. From the retention results, it can be inferred that IT cortex is involved in pattern-from-motion discrimination in the normal monkey. From the acquisition results, it would appear that surviving cortex can support normal learning of pattern-from-motion, which is not the case for the learning of pattern-from-luminance. (Supported by NEI grant EY5603)

497.7

COMPUTER SIMULATIONS OF MULTIPLE, INTERCONNECTED VISUAL CORTICAL AREAS: FUNCTIONAL INTEGRATION AND ILLUSORY RESPONSES. L.H. Finkel. The Neurosciences Institute and The Rockefeller University, New York, NY 10021.

A large-scale computer simulation of the magnocellular pathway in areas V1, V3, and V5 (MT) of macaque visual cortex has been used to study the mechanisms of integration of distributed cortical networks. The simulated networks contain over 220,000 neuronal units and 8 million connections. Physiological properties of units in each simulated area correspond to those recorded in vivo, including directional selectivity in simulated area V5 similar to that described by Movshon et al. (Exp. Brain Res. Suppl. 11:117, 1985).

Visual stimuli are presented and network responses are compared to reported physiological and psychophysical observations. The integrative actions of the system are most clearly revealed by responses to several visual illusions including illusory contours (IC's), structure-from-motion (SFM), and a novel combined illusion which uses SFM to generate illusory contours. The same network architecture discriminates occlusion boundaries, IC's, and SFM by performing the same neuronal operations on inputs from different areas.

The simulations provide a framework for studying possible mechanisms of cortical integration and generate a series of testable experimental predictions.

497.8

VISUAL MOTION ANALYSIS WITH IMPAIRED SPEED PERCEPTION: PSYCHOPHYSICAL AND ANATOMICAL STUDIES IN HUMANS. L.M. Vaina, M. LeMay*, A. Choi*, T. Kemper* and D. Bienfang*. Biomed. Eng. and Neurology Dept., Boston Univ., Harvard-MIT Div. of Health Sci. & Tech., and Harvard Med. Sch., Boston, Ma 02215.

Over the past several years we have studied numerous patients with specific psychophysical deficits of motion analysis due to local lesions to the visual cortex. Lesions were unilateral and were localized by CT studies. Here we report results from a series of psychophysical motion tasks in patients with bilateral lesions involving the visual cortex. First, we assessed performance on tasks involving color, form, binocular stereopsis, contrast, texture and motion discrimination. Secondly, we investigated ability to discriminate speed, to detect boundary and form from relative motion, to recover 3-D structure from motion, and to perceive motion coherence. To minimize position cues, we used dynamic random dot displays, varying dots' lifetime and density. Patients' performance suggests that (a)contrast sensitivity and discrimination of form, color, or texture can be decoupled from perception of speed; (b)perception of speed of motion can be decoupled from ability to recover structure from motion and from ability to perceive coherent global motion from random local motion fields; and (c)detection of discontinuities in motion can be dissociated from the computation of motion and interpretation of global speed fields.

In conclusion, we find a specific correlation between deficits of motion analysis and the anatomical locus of lesions. On the basis of the specific anatomical locus of lesion, the pattern of myelination seen in the MRI scans, and the putative location of the area MT in the human brain (from PET studies), we suggest that the lesions in two of our subjects might involve the human homologue of MT, extrastriate visual area which is believed to be involved in processing information critical for perception of visual motion. These subjects were impaired on speed discrimination and perception of motion coherence. Stereopsis was lost in both patients. They were able, however, to detect 3-D structure from motion, and conrast, form, and color discrimination were normal.

497.9

Spatial Memory and the Accuracy of Saccades to Remembered Visual Targets. P. Moeller, M. Hayhoe, D. Ballard, J. Albano, (SPON: W. Makous). Center for Visual Science, University of Rochester, Rochester NY.

This experiment investigates the role of relative position information in guiding saccades to remembered targets. Stimuli were presented in the dark on an oscilloscope with a P31 phosphor, viewed through a long wave cut-off filter to minimize persistence. Subjects maintained fixation while two test targets were presented *simultaneously*. The test targets were extinguished and after a dark interval the *trigger* target reappeared in one of the two previously presented test locations. This cued subjects to make a saccade first to the center of the visible trigger target, and then to the center of the previously displayed *memory* target. In a second condition, the trigger target was presented *sequentially* after presentation of the memory target. In the first condition, the spatial relationship between the two targets is made available. In the second condition it must be computed from information made available at different times. In both conditions targeting accuracy was good compared with visual targets. However performance was substantially better in the simultaneous condition. This result suggests that the spatial relationship between simultaneously appearing targets is stored in memory and is used to improve the accuracy of saccades to targets when they are no longer visible. In a second experiment, an extra saccade was interposed between target presentation and the trigger. This had little effect on error in either simultaneous or sequential condition. This suggest that unmonitored drift in the dark intervals is the only additional source of error in the sequential condition.

Supported by EY01319, EY05729, and EY07344.

497.10

ORIENTING TO NEGLECTED HEMISPACE DEPENDS ON THE GEOMETRY AND SALIENCE OF THE DISPLAY. M. Rizzo and R. Hurtig. Div. of Behavioral Neurology and Cognitive Neuroscience, Univ. of Iowa College of Med., Iowa City, Iowa 52242.

Humans with unilateral cerebral lesions may fail to explore contralateral visual hemispace. We had an opportunity to observe how such defects may depend on properties of the stimulus under consideration.

We studied 3 subjects with CT/MR verified unilateral hemisphere lesions. Hemineglect was behaviorally evident on tasks such as line cancellation. Eye movements were recorded using EOG (DC coupled, bandwidth 0-35 Hz, resolution 0.5 deg). The subjects showed deficient tracking of suprathreshold light targets into the affected hemispace; one subject completely failed to follow any target directed to the left. When we recorded visual scanning of picture scenes and faces performance was improved. Recruitment of fixation into the aberrant hemifield depended on feature density, continuity, and stimulus salience. Faces, which are frequently encountered objects of high signal value, were more likely to engage scanning across the midline.

Results suggest that visual orienting into neglected hemispace depends on: (1) bottom-up factors related to geometrical closure, scale, symmetry, and the formation of conjunctions among contiguous features, and (2) top-down factors related to previous knowledge or biological salience of target displays.

497.11

MEASURING FACIAL EXPRESSIONS. C.M. Leonard, Dept. of Neuroscience, Univ. Fl. College of Medicine, Gainesville, FL 32610.

Facial expressions are nonverbal communication signals that primates use to signal their feelings, desires and intentions. Although neurons responsive to these signals have been found in the temporal lobe they are difficult to investigate because of the complexity of the effective stimuli. The goal of the present experiments is to develop methods for evaluating facial expressions in humans that could be adapted to study the neural encoding mechanisms in animals.

Initial observations were made on 8 videotaped smiles by 4 women. Twelve sequential 33ms frames were digitized from each smile. The pixel gray levels from successive frames were subtracted so that the difference between frames could be expressed quantitatively, as mean, entropy and variance. We found that different individuals had smiles with different temporal patterns of variance and entropy change. Some smiles accelerated and decelerated rapidly, while others changed at a constant high or low rate. Subjective ratings of smile quality paralleled the objective measures of variance and entropy. Raters (n = 7) found that high variance smiles changed quality more rapidly than low variance smiles (r = .60).

Facial expressions are easier to detect and identify when they are moving. Therefore we used movement detection in a two-alternative forced-choice paradigm as a nonverbal measure of expression salience. Changes in high variance smiles were easier to detect than changes in ones with low variance. Contrast sensitivity functions can determine the relative detectability of expressions by different individuals in different contexts.

Neurophysiological experiments with monkey facial expressions can explore whether contrast sensitivity functions predict neural firing rates. Detection accuracy values greater than predicted by the objective measures of variance and entropy might indicate activation of neural feature detectors selective for facial expression.

REGENERATION II

498.1

NERVE REGENERATION THROUGH SILICONE TUBES IN$ MONKEYS. C.B.Jeng and S.H.Jou*. Dept. Anat., Chang Gung Med. Col., Tao-yuan, Taiwan 33333.

In the present study we asked if a synthetic tube guide without any peripheral nerve components allows primate nerve to regrow over a long distance. Under deep nembutal anesthesia, the tibial nerves were sterilely exposed at the thigh and transected twice to remove a 2.0-2.5cm nerve segment in adult monkeys(Macaca cyclopis). The resulted nerve stumps were anchored with a 9-0 prolene stitch in silicone tubes (11FR, Goleta, CA) to maintain a 3cm or 4cm interstump gap. Five weeks after surgery, there was a nerve cable connecting the nerve stumps. At twelve weeks numerous nerve fascicles containing many unmyelinated axons and few myelinated axons were seen at the mid of the cable and the distal end of the tube. We therefore conclude that regenerating axons are able to grow across a 4cm distance without an aid of autologous nerve graft in monkeys.(Supported by the American Muscular Dystrophy Association, CMRP260 of Chang Gung Med. Col. and NSC78-0412-B182-11 of R.O.C.)

498.2

COMBINED USE OF EMBRYONIC NEURAL TISSUE AND SYNTHETIC POLYMER TUBES TO PROMOTE CNS AXON REGENERATION. M. Kliot☆ A. Kader, R. Riso☆ P. Brittis, P. Aebischer* and J. Silver☆ Neurological Institute, NYC, NY 10032 and Case Western Reserve Univ. School of Medicine, Cleveland, Ohio 44106.

Recently several strategies have succeeded in promoting the regeneration of dorsal root axons into the adult mamalian spinal cord. Using Millipore implants coated with embryonic astrocytes, we induced the regrowth of injured dorsal root fibers into the rat spinal cord with the formation of axon terminals. Regeneration was enhanced by the avoidance of white matter through the implantation of cut dorsal root fibers into the dorsal horn grey matter (Siegal et al.,1988). Examples of regeneration, however, occurred in only about 25% of the animals.

To further define and enhance conditions promoting axon regeneration, we are employing acrylic polymer tubes as conduits for regrowing dorsal root fibers. We have been able to demonstrate histologically that dorsal root fibers can grow successfully within these tubes. We are currently investigating their capacity to promote the ingrowth of dorsal root fibers into the adult spinal cord. These tubes are implanted into the dorsal horn either alone or in combination with spinal cord taken form embryonic day 12-14 day old rats.

498.3

REGENERATION OF ADULT DORSAL AND VENTRAL ROOT AXONS IN SEMIPERMEABLE GUIDANCE CHANNELS. M. McCormack*, V. Guénard, M. Goddard*, A. Beauregard*, S. Brace*, P. Aebischer (SPON: K.Thrivikraman). Artificial Organ Laboratory, Brown University, Providence, RI 02912.

The use of semipermeable guidance channels has been reported to provide a more appropriate regenerating environment by allowing solute exchange across the channel's wall. In the present study we evaluated the ability of semipermeable guidance channel to support regeneration of both dorsal and ventral roots. In one cohort the L5 dorsal root (n=3) was transected, a 2 mm segment excized and an entubulation repair with an acrylic copoplymer semipermeable guidance channel (50 kDa Mw cut-off) was performed leaving a 4 mm gap. The same procedure was performed on the L5 ventral root in a second cohort (n=3). Following a 6 month implantation interval, HRP labeling of the dorsal root lesioned animals was performed bilaterally via sciatic nerve transections at the mid thigh. The ventral root lesioned animals were labeled by applying HRP on the L5 spinal nerve exiting the root canal. Number of myelinated axons was determined at the channel midpoint. All channels contained a regenerated nerve cable bridging both nerve stumps. The number of myelinated axons regenerated in the ventral roots was significantly greater than that observed in the dorsal roots. The ratio of regenerated ventral root axons versus control equaled 2.9 whereas that of dorsal root equaled 0.3. No significant difference in number of HRP labeled cells was observed in either ventral horn or dorsal root ganglia neurons between the experimental and control side. This study suggests a different regenerative capacity between ventral and dorsal root axons.

Supported by NIH NS 26159.

498.4

REQUIREMENT FOR A 1-μm PORE CHANNEL OPENING DURING PERIPHERAL NERVE REGENERATION THROUGH A BIODEGRADABLE CHEMICAL ANALOG OF ECM. I.V. Yannas, A.S. Chang*, S. Perutz*, C. Krarup*, T.V. Norregaard*, N.T. Zervas. Fibers and Polymers Lab., Mass. Inst. of Tech., Cambridge, MA 02139; Division of Neurology, Brigham and Women's Hospital, Boston, MA 02115; The New England Deaconess Hospital, Boston, MA 02115; and Division of Neurosurgery, Mass. General Hospital, Boston, MA 02115.

A continuing study of preferences of elongating axons and Schwann cells for specific matrix features has revealed two apparently critical requirements. Well-defined, chemical analogs of ECM based on a collagen-glycosaminoglycan (CG) copolymer were used to bridge a 10-mm gap between cut ends of the rat sciatic nerve. The nerve stumps and the CG matrix bridging them were ensheathed in a silicone tube. Electrophysiological properties of regenerating motor nerve fibers innervating the plantar flexor muscles were serially monitored over 40 weeks following surgery. The results suggest that functional recovery of motor function requires the presence of a) a rapidly degrading CG copolymer matrix and b) an average pore diameter of order 1 μm. These results pose novel questions about the nature of cell-ECM interactions during regeneration.

Supported by NSF Grant EET-8520548.

498.5

BASIC FGF-CONDITIONED IMPLANTS SUPPORT *IN VIVO* GROWTH OF TRANSECTED RETINAL AXONS IN YOUNG HAMSTERS. L.S.Carman* and G.E.Schneider.(SPON: D.J.Ingle) Dept. Brain and Cognitive Sci., M.I.T., Cambridge,MA 02139.

Basic fibroblast growth factor is a potent mitogen for a wide variety of neural and non-neural cells. In addition, bFGF enhances survival and neuritic outgrowth of neurons from many CNS regions *in vitro*. We examined the *in vivo* effect of bFGF on injured retinal ganglion cells in the neonatal hamster by placing bFGF-conditioned implants adjacent to transected retinal axons, which normally will not regenerate if transected on postnatal day 4 (p4) or later. For hamsters receiving optic tract transection on p6, 10, 18, and 25, regenerative growth into the implants was significantly greater than in saline-conditioned controls. In the p6 group, the number of surviving retinal ganglion cells did not differ between lesioned-bFGF, lesioned-saline, and unlesioned cases. Survival was not measured at other ages.

Because regenerating axons grew preferentially on GFAP+ cells and processes, we suggest that enhanced retinal axon outgrowth results from bFGF induced astrocytic proliferation, rather than from direct neurite-promoting effects. Basic FGF-induced astrocytic proliferation may enhance formation of a glial substrate that supports regrowing retinal axons. [Supported by NIH grants EY00126, EY02621, NIGMS T32-GM07484. Research conducted in compliance with NIH Publ. No. 85-23, 1985.]

498.6

NERVE GROWTH FACTOR ENHANCES EARLY REGENERATION OF RAT MYELINATED AXONS THROUGH SILICONE CHAMBERS. K.M. Rich, J.C. Pryor*, and J.P. Hollowell*. Departments of Neurosurgery and Neurobiology, Washington University Sch of Med, St. Louis, MO 63110.

The influence of NGF on regeneration of myelinated axons across a gap in adult rat sciatic nerve was examined to determine whether NGF specifically enhances sensory and/or motor axonal growth. Either dorsal root ganglionectomies (DRG_x) or ventral rhizotomies (VR_x) at L_{4-5} were performed on Sprague-Dawley rats. The rats underwent ipsilateral sciatic nerve section and surgical implantation of a silicone chamber with an 8-mm gap between proximal and distal nerve ends. The chambers were filled with a sterile solution of either 1 mg/ml NGF (experimental) or normal saline (control). Four weeks after surgery, semi-thin cross-sections were prepared and stained with toluidine blue. Myelinated axonal counts were determined from both the proximal and distal ends within the chamber of the regenerated nerve. Thin sections were prepared for ultrastructural examination. After the DRG_x and the VR_x there were mean counts of 2859 (motor) and 6794 (sensory) myelinated axons in the sciatic nerve proximal to the chamber in the respective groups. In the NGF- treated VR_x group within the chambers, there were proximally 4712 ± 808 and distally 1607 ± 368 sensory myelinated axons, compared to the control group with proximally 3236 ± 293 and distally 879 ± 238 sensory myelinated axons. In the NGF-treated DRG_x group, there were proximally 388 ± 92 and distally 77 ± 56 motor myelinated axons compared to the control group with proximally 327 ± 118 and distally 32 ± 13 motor myelinated axons. Although the motor myelinated axons did not grow across the chambers in significant numbers in either the NGF or the control groups, ultrastructural examination demonstrated numerous unmyelinated axons in regenerative units in the distal portions of the DRG_x chambers. Thus, NGF enhanced early regeneration of sensory myelinated axons through a silicone chamber four weeks after nerve section. NGF had no effect on motoneuron regeneration in this experimental paradigm.

498.7

DISSOCIATED SCHWANN CELL/EXTRACELLULAR MATRIX TRANSPLANTS ENHANCE CNS AXONAL REGENERATION. L. F. Kromer and C. J. Cornbrooks, Dept. of Anat. & Cell Biol., Georgetown Univ., Washington, DC 20007 and Dept. of Anat. & Neurobiol., Univ. of Vermont, Burlington, VT 05405

Prior experiments using transplants of intact Schwann cell-neurite units obtained from long term dorsal root ganglion (DRG) explants demonstrated that this in vitro preparation could foster cholinergic axonal regeneration between the septum and hippocampus. The objective of this study was to further evaluate whether the three-dimensional organization of this preparation, which contained miniature bands of Bungner, was essential for promoting this regeneration response. For these experiments Schwann cells were dissociated either from DRG cultures or from monolayer cultures obtained from neonatal rat sciatic nerve and then mixed with tumor-derived extracellular matrix (ECM). This material was allowed to form cell/matrix cables within selectively permeable polymer tubes that were transplanted into a lesion of the septo-hippocampal pathway. Regeneration of cholinergic axons into the cell/matrix cable was evaluated 1-8 weeks posttransplantation. Within one week Schwann cells migrated to the surface forming a cylinder along the length of the matrix cable. Cholinergic axons were observed to rapidly regenerate along this Schwann cell cylinder but very few axons were found in the ECM core of the cable. The rate of axonal growth was as rapid as that observed on the entact transplants containing the miniature bands of Bungner suggesting that preformed basal lamina tubes containing Schwann cells are not necessary to enhance CNS axonal regeneration through these transplants.

498.8

NGF CAN REPLACE SCHWANN CELLS IN PERIPHERAL NERVE GRAFTS USED FOR CENTRAL NERVOUS SYSTEM REGENERATION. M. Manthorpe, T. Hagg, A.K. Gulati*, A.M. Behzadian*, H.L. Vahlsing*, S. Varon. Dept. Biology, M-001, UCSD, La Jolla, CA 92093.

Adult mammalian CNS axons readily regenerate through peripheral nerve grafts containing viable Schwann cells but not as well through freeze-thawed, non-viable nerve grafts. One possibility is that living Schwann cells within the grafts contribute neuronotrophic factors like nerve growth factor (NGF) which attract and promote elongation of incoming central axons. We compared CNS cholinergic axonal regeneration through freshly dissected peripheral nerve grafts with that occurring through "acellular" PNS grafts, free of Schwann cells and debris, which had been treated with or without NGF. Acellular sciatic nerve grafts were prepared by 6 week pre-degeneration in situ, freeze-thawing, removal of cellular debris by incubation for 7 days in vitro with macrophages, freeze-thawing again and finally incubation with saline or saline plus purified β-NGF. Two mm pieces of fresh autologous or acellular nerve were placed between bilaterally disconnected septum and hippocampal formation of adult female Sprague-Dawley rats. After one month, non-NGF treated acellular grafts contained a modest number of parallel AChE-positive fibers. NGF-treated grafts contained many more fibers and such fiber growth was comparable to that seen with fresh autologous control grafts. These results indicate that i) acellular peripheral nerve grafts can act as a terrain for CNS axonal regeneration and ii) treatment of the grafts with NGF before implantation allows them to perform as well as viable cellular nerve grafts. This suggests that, at least for CNS cholinergic axonal regeneration, Schwann cells in peripheral nerve grafts can be replaced by NGF. Supported by grants NIH NS-25011 and NSF BNS-88-08285.

498.9

CHOLINERGIC SEPTO-HIPPOCAMPAL REGENERATION THROUGH SCIATIC NERVE BRIDGES : QUANTITATIVE TEMPORAL DEVELOPMENT T. Hagg, H.L. Vahlsing*, S. Varon, M. Manthorpe. Dept. Biol., UCSD, La Jolla, CA 92093.

Vigorous axonal regrowth can occur from various CNS regions into transplanted adult PNS or fetal brain tissue. However, regrowth from the graft into adult host CNS tissue is limited. Here we have examined the temporal pattern of cholinergic re-innervation of rat hippocampi through pieces of viable autologous sciatic nerve placed between bilaterally disconnected septum and hippocampal formation of adult female Sprague-Dawley rats. At different times after implantation, brains were paraformaldehyde-fixed, cut up to a level just rostral to the fornix in a coronal plane, and the rest of the brain cut in a sagittal plane. This approach allowed for concurrent standardized evaluation of the septal cholinergic neurons and analysis of the re-growing fibers in a plane along their general orientation. Cholinergic axonal regeneration was quantified in sagittal sections throughout the brain by counting the number of acetylcholinesterase (AChE)-positive fiber intersections at successive 1 mm distances caudal to the rostral tip of the hippocampal formation. These numbers were furthermore recalculated into single composite values expressing the amount and extent of regeneration.

After one week no or very few AChE-positive fibers were detected in the graft. By week two large numbers of fibers were seen within the nerve bridge reaching the hippocampal interface. From 2 weeks post-implantation onwards the entry and advance into the hippocampal formation proceeded slowly but consistently. By 1, 2 and 6 months substantially more fibers with a more extensive distribution were detected and the number of fibers in the dorsal 1-2 mm of the dentate gyrus reached normal levels. At all time points the amount of regeneration was several-fold higher than that seen with gelfoam-implanted controls. This temporal baseline of cholinergic regeneration will allow future quantitative comparisons of the effects of different manipulations, e.g. NGF treatments, or different bridge material. Supported by grants NSF BNS-88-08285 and NINCDS NS-25011.

498.10

SERINE PROTEASE INHIBITORS BLOCK POSTRANSLATIONAL MODIFICATION OF PROTEINS BY ARG AND LYS. M. Yu*, G. Chakraborty*, D. Luo* and N. Ingoglia. Dep't of Physiology, New Jersey Medical School, Newark, N.J.

The posttranslational incorporation of amino acids into proteins has been demonstrated in axons and supporting cells of a variety of neural tissues (Ingoglia et al., in Axonal Transport, Alan R. Liss Inc., 1987; 435). These reactions are regulated by endogenous molecules which must be removed from the reaction components in order for protein modifications to occur. In the present experiments we have attempted to characterize these molecules in brains and regenerating sciatic nerves of rats. Of a variety of exogenous substances examined, we have found that two serine protease inhibitors, bovine pancreatic trypsin inhibitor (BPTI, MW = 6K) and alpha 2 macroglobulin are powerful blockers of these reactions, whereas inhibitors of calcium activated proteases, metaloendoproteases, thiol proteases and PMSF have little or no activity. Since the endogenous regulator of these reactions is a heat stable, 1-10kd peptide which can be inactivated by trypsin, we propose that the endogenous regulator is a serine protease inhibitor with BPTI-like characteristics. Supported by grants NS 19148 and EY06728 from NIH.

498.11

APOLIPOPROTEIN D ACCUMULATES IN THE REGENERATING PERIPHERAL NERVE. J. K. Boyles, L. M. Kosik*, and M. R. Wardell*. Gladstone Foundation Laboratories, Dept. of Pathology and CVRI, Univ. of California, San Francisco, CA 94143.

Massive quantities of lipids are required to form the new axonal membranes and myelin of regenerating peripheral nerves. Earlier, we showed that apolipoprotein (apo-) E produced by local macrophages and apo-A-I entering from the plasma, together with LDL receptors expressed by axons and Schwann cells, could provide a mechanism for the necessary lipid transfers. We have now isolated and identified another protein produced by the cells of the injured rat sciatic nerve, apo-D. Apolipoprotein D has been identified previously only in primates, where it is found on plasma lipoproteins. We have isolated apo-D from the lipoprotein particles present in regenerating rat sciatic nerves. It is not present, however, on the lipoproteins from rat plasma. We identified the nerve apolipoprotein as apo-D on the basis of amino acid sequence homology with human apo-D. Its amino acid composition, isoelectric point, molecular weight, and glycosylation pattern were also found to be similar to those of the human protein. Immunocytochemical studies of tissues from the rat central and peripheral nervous systems indicated that apo-D is a product of specific neurons and glial cells, and that its production increases during regeneration. Western blots of extracts from regenerating sciatic nerves confirmed that apo-D increases in concentration severalfold during the first day following a crush injury, peaking at 3 weeks when it is increased 350-fold. Thus, neurons and glial cells responding to injury increase expression of apo-D, a protein that may participate in the lipid transfer processes of regeneration.

498.12

CALCITONIN GENE-RELATED PEPTIDE IN THE REGENERATING CENTRAL AND PERIPHERAL NERVOUS SYSTEM. F.L.Dumoulin*, G.Raivich*, W.J.Streit and G.W.Kreutzberg. Department of Neuromorphology, Max-Planck-Institut für Psychiatrie, D-8033 Martinsried, F.R.G..

We have studied the distribution and content of the putative neuromodulator calcitonin gene-related peptide (CGRP) and its receptor in the regenerating central and peripheral nervous system. Axotomy of the rat facial nerve leads to a striking increase in CGRP-immunoreactivity of parent motorneurons, which is most pronounced in stem dendrites. CGRP-radioimmunoassay (RIA) showed the increase to be already present at day one after axotomy, reaching a maximum of about 300% at day 5, then slowly decreasing. The regenerating rat sciatic nerve showed a slight depression of anterograde axonal transport of CGRP recovering around the time of reinnervation as demonstrated by RIA. We also see strong autoradiographic labelling of CGRP binding sites occurring in a spatio-temporal pattern very similar to that of axotomy induced blood-nerve-barrier deficit.
In conclusion we suggest that a) CGRP might be a mediator of neuronal-glial interaction during retrograde reaction in the facial nucleus after axotomy; b) CGRP, which is known to be vasoactive, may have a function in the regulation of perfusion and permeability in the regenerating peripheral nerve; c) as a known antisprouting agent CGRP might participate in regulatory processes during regeneration of both sensory and motor systems.
1. Streit, W.J. et al., Neuroscience Lett. 1989 (in press).

498.13

PLATELET DERIVED GROWTH FACTOR AND CENTRAL NERVOUS SYSTEM REGENERATION. M. Lotan*, A. Cohen*, R. Duvdevani* and M. Schwartz. Dept. of Neurobiol. Weizmann Inst. science, Rehovot, Israel

Recent studies indicate that during development, platelet-derived growth factor (PDGF) is involved, in regulating the differentiation of oligodendrocytes from their progenitors. The physiological significance of PDGF in the adult central nervous system (CNS) following injury is provided in the present study. The visual system of fish and rat have been used as models for regenerative and non-regenerative CNS, respectively. Conditioned media (CM) from normal/injured rat and fish optic nerves were assayed for presence of PDGF by both mitogenic activity (using 3T3 fibroblast cell line) and immunoreactivity (using rabbit anti-human PDGF-AA polyclonal antibodies in radioimmunoassay). In fish CM, PDGF-like activity was higher in the non-injured nerve as compared with the injured nerve. In rat CM, high levels of PDGF were detected in the newborn optic nerve, while no activity was observed in the adult nerve. However, following injury, already at day one there was a 5 fold increase in PDGF levels which started to decline by day 7. We conclude:1.The role of PDGF in development and regeneration is different, and therefore the regulation of oligodendrocytes differentiation during regeneration is performed via an additional factor(s). 2.Elevation of PDGF levels following injury might interfere with regeneration.

498.14

REGENERATION OF ADULT OPTIC NERVE IS ASSOCIATED WITH OLIGODENDROCYTE MATURATION INHIBITORY FACTOR. A.Cohen*, T.Sivron* R.Duvdevani* and M.Schwartz. (SPON:C.Stein-Izsak). Dept. of Neurobiol. Weizmann Inst. of Science, Rehovot, Israel.

Lack of regeneration in the mammalian central nervous system (CNS) has been attributed to a non-supportive and non-permissive glial environment. We previously showed that factor(s) in conditioned media (CM) from growing/developing nerves (mammalian CNS or regenerating fish optic nerves), can cause anatomical regeneration in a non-regenerative system, possibly due to changes in the environment. We now show that CM from regenerating fish optic nerves include factor(s) which inhibit oligodendrocyte maturation from their progenitors. Primary cultures of glial cells from injured adult rat optic nerves or oligodendrocytes from newborn rat brains were used. The addition of CM of regenerating fish optic nerves to these cultures inhibited maturation of oligodendrocytes, evident morphologically and immunocytochemically using antibodies specific to mature and premature oligodendrocytes (galactocerebroside, (Galc) and O-4, respectively). CM from non-injured fish optic nerves lacked this inhibitory activity. Treatment of the cultures with platelet derived growth factor resulted in an apparent reduction in the number of $Galc^+$ cells, but in an increase in their progenitors ($A_2B_5^+$). Successful regeneration in the CNS appears to be associated with factor(s) which inhibit maturation of oligodendrocytes thereby possibly maintaining an environment permissive for axonal growth.

TROPHIC INTERACTIONS I

499.1

DOES REINNERVATION WITH ELECTRICALLY SILENT AXONS AFFECT THE EXTRAJUNCTIONAL MEMBRANE PROPERTIES OF DENERVATED SKELETAL MUSCLES? A. Cangiano, M. Buffelli* and E. Pasino*. Istituto di Fisiologia Umana, Università di Verona, Italy.

It has been repeatedly shown that muscle disuse affects the extrajunctional membrane properties to a smaller extent than denervation does. In the special case of disuse represented by reinnervation with electrically silent axons, partial recovery of the alterated membrane properties has been, accordingly, reported. These results can support the existence of neural impulse-independent factors having a muscle regulatory action, but they can alternatively be explained by incompleteness of the conduction block utilized. To improve the block, we tested various concentrations of the blocking agent (TTX) and applied it to the entire circumference of the treated nerve. The drug was chronically delivered from Alzet minipumps to appropriate silastic cuffs implanted around the sciatic nerve of anesthetized rats. Simultaneously, the nerves of the ipsilateral soleus and EDL muscles were crushed. A few days later reinnervation begun, however without return of activity. The contralateral muscles were also denervated but their reinnervation prevented.
After 15-35 days, soleus and EDL muscles were isolated in vitro and extrajunctional properties (resting potential, acetylcholine receptors, action potential sensitivity to TTX) were measured. No quantitative differences between reinnervated impulse-blocked and denervated muscles were detected, provided high dosages (> 8-9 µg/day) of TTX had been used. These results were not due to systemic actions of TTX; they were only obtained when the nerve dose and not the systemic dose was high. We conclude that impulse activity is a prerequisite for the return towards normality, in any measurable amount, of the extrajunctional membrane properties of denervated muscle upon reinnervation.

499.2

ANALYSIS OF MOLECULAR FACTORS SECRETED BY INJURED FROG SCIATIC NERVE. B. Tedeschi* (SPON: D. Meyer). Dept. of Anatomy & Cell Biol., Eastern Virginia Medical School, Norfolk, VA 23501.

At least three polypeptides (30kD, 35kD, and 70kD) have been found to be secreted in significantly greater amounts from regenerating bullfrog sciatic nerve (Tedeschi, B. and Wilson, D.L., J. Neurochem., 48:463, 1987). In order to determine whether such secreted sciatic nerve factors might have a trophic or developmental role, PC12 cells were cultured in media (RPMI-10% horse serum/5% FCS), with or without Nerve Growth Factor (NGF), that was supplemented with frog sciatic nerve conditioned fluid (FSN-CF). Media supplemented with FSN-CF did not elicit differentiation and neurite outgrowth in PC12 cells grown without NGF. However, FSN-CF-supplemented, NGF-containing media enhanced the amount of neurite outgrowth of PC12 cells relative to that obtained in unsupplemented NGF-containing media, suggesting the presence of a growth or trophic factor(s) in FSN-CF. In order to further characterize the important functional molecular components of FSN-CF, a specific polyclonal antibody against the secreted 70kD polypeptide has been developed. Further functional analysis of the 70kD FSN-CF polypeptide will be discussed. This work was supported by NSF (BNS-8607811) and EVMS BRSG grants.

499.3

THE EFFECTS OF CALCIUM ON GANGLIOSIDE-MEDIATED NEURITOGENESIS. P.E. Spoerri, A.K. Dozier*, C.G. Caple* and F.J. Roisen. Dept. of Anatomical Sciences & Neurobiology, Univ. of Louisville, Sch. of Med., Louisville, KY 40292.

Considerable evidence suggests that gangliosides, especially GM1, play a role in the development of the nervous system. Previously, we have shown that exposure of murine Neuro-2a cells to GM1 resulted in dramatic neuritogenesis. Furthermore, we and others have demonstrated that GM1 potentiated the action of NGF and NGF-independent trophic factors on a variety of primary neurons in vitro. The mechanism through which gangliosides function is unknown. To determine if ganglioside stimulation of neuritogenesis is dependent on calcium, we evaluated the effects of altered intracellular calcium levels on GM1-mediated sprouting and elongation of Neuro-2a cells. These studies employed Ca^{+2} ion blockers (Cd^{+2}, La^{+3}, ruthenium red), EGTA, A23187, taurine and calcium adjusted medium. The resultant neuritogenesis was quantitated microscopically with computer-assisted morphometry and ultrastructurally with electron microscopy. The neuritogenic activity of GM1 was enhanced by A23187, taurine and high extracellular Ca^{+2} and diminished by agents which reduced intracellular Ca^{+2}. We are employing x-ray microprobe analysis to localize Ca^{+2} in Neuro-2a cells after GM1 treatment. These studies suggest that the action of GM1 on Neuro-2a cells is calcium dependent. USPHS grants NS24524 and DE07734 to FJR.

499.4

SOLUBILIZED MUSCLE MEMBRANE FRACTION REGULATES MOTORNEURON ADENYLATE CYCLASE, F-ACTIN AND ACH SENSITIVITY. J.B. Tuttle Neuroscience, Univ of Virginia Hlth Sci Cntr, Charlottesville, VA 22908

Ciliary ganglion neurons from 11d avian embryos lose responses to acetylcholine (ACh) over 3-4 days of solitary cell culture, while neurons synapsing with cultured myotubes or on lysed muscle membranes retain ACh sensitivity. This suggests molecule(s) in the muscle membrane regulate neuronal ACh sensitivity via a retrograde contact interaction. Treatment of lysed myotube membranes with TRITON-X 100 (0.5%) or high or low salt did not remove the retrograde activity, while deoxycholate did. A deoxycholate solubilized membrane fraction cross-linked to the culture substrate maintained high levels of neuronal ACh sensitivity. Other mesenchymal cells do not contain similar activity, suggesting specificity to muscle. The activity has no survival-promoting action and does not co-immunoprecipitate with NCAM. This indicates a contact interaction between these peripheral neurons and components in the target membrane is a specific signal influencing ACh sensitivity. Culture on the factor-linked substrate or on muscle membranes down-regulates neuronal cAMP accumulation during IBMX diesterase blockade, with and without forskolin stimulation. Submembranous F-actin co-localizes with immunoreactive ACh receptors in these neurons, and the membrane factor increases actin polymerization in culture. These results suggest neuronal contact interaction with the target membrane has profound regulatory consequences to receptor function, cytoskeletal organization and intracellular metabolism.

499.5

DEVELOPMENTAL INTERACTIONS OF BASIC FGF AND SCHWANN CELL EXTRACELLULAR MATRIX WITH CILIARY GANGLION NEURONAL SURVIVAL IN CULTURE. *Ken Vaca*, Dept. of Neurology, Baylor College of Medicine, Houston, TX 77030.

Developmental changes in the time course of neuronal survival were examined in cultures of dissociated chick ciliary ganglia from embryonic days (E)6 to E13. Cells were plated in poly-lysine coated 35mm dishes at an initial density of about 12,000 neurons/plate. Neurons from E6 and E7, prior to the onset of synaptic transmission with muscle and normal cell death, experience a rapid initial die-off, which is substantial after 1 day in culture and essentially complete after 3 days. This rapid loss is only partly prevented by the addition of basic FGF, a neurotrophic factor produced by muscle. The neuronal loss is slower in cultures from E8 and E9 and by E9 can be almost totally prevented by the addition of basic FGF. By E13, at the end of the cell death period, about 25% of the neurons survived in control cultures after 15 days, and all loss was prevented by basic FGF. At E6 and E7, neurite outgrowth was very rare, except when FGF was added. From E9 to E13, some control neurite outgrowth was more common, but was substantially enhanced by FGF.

It was noted that the initial plating density of the indigenous Schwann cells increased with the age of the ganglia even as the neuronal plating density was kept constant. Schwann cell enriched cultures were prepared from oculomotor nerves of E7 or E13 embryos. Conditioned medium from cultures of either E7 or E13 Schwann cells had relatively little effect on neuronal survival, even when Schwann cells were grown in the presence of basic FGF, which was then removed by heparin affinity chromatography. Extracellular matrix of Schwann cell cultures, prepared by lysis with Triton X-100, dramatically increased neuronal survival from E7 ganglia, but had relatively little effect on neurite outgrowth. This increase in survival was synergistically enhanced by basic FGF.

499.6

NGF MEDIATES BASAL FOREBRAIN NEURON-SUPPORT CELL INTERACTION IN CULTURES. M. Yokoyama, B. Lu, I.B. Black and C.F. Dreyfus. Div. Devel. Neurol., Cornell Univ. Med. Coll., New York, N.Y. 10021.

Previous studies indicate that NGF and non-neuronal cells from the basal forebrain (bf), hippocampus (hi), and cerebellum (cb) stimulated the acetylcholine synthetic enzyme, choline acetyltransferase (CAT), in bf neurons. The present work was designed to investigate the role of NGF in mediating non-neuronal cell effects.

We initially determined whether the non-neuronal populations expressed NGF mRNA. Non-neuronal cells were grown to confluency and NGF mRNA was measured using a sensitive nuclease protection assay. In fact, NGF mRNA was detected in cultured non-neuronal cells from the bf, hi, and cb, suggesting that NGF is synthesized by diverse populations and plays a role in the bf-support cell interaction.

To further define the action of NGF in the bf system, addtional experiments delineated effects of the trophic agent on development of bf neurons in culture. Virtually pure population of dissociated bf neurons were maintained in the continuous presence of NGF or control medium. CAT activity was used to monitor responsivity to NGF. Bf neurons exhibited a marked enhanced response to NGF with time in culture. Thus cells grown for 7 days in NGF exhibited a 2-fold increse in CAT activity, while cultures grown for 10 days demonstrated a 4-fold increase. To begin to determine whether increased responsiveness was due to the level of NGF receptor expression, development of receptor mRNA was measured. Bf cultures exhibited a marked increase in NGF receptor mRNA levels, corresponding to elevated responsiveness to NGF. Consequently the enhanced effects of NGF may be directly related to increased numbers of NGF receptor expression. (Support: NIH grant HD 23315, NS 10259)

499.7

FIBROBLAST GROWTH FACTOR PROTECTS AGAINST GLUTAMATE-INDUCED DEGENERATION IN HIPPOCAMPAL NEURONS. M. P. Mattson[1], M. Murrain[2], P. B. Guthrie[2] and S. B. Kater[3]. [1]Sanders-Brown Center on Aging, Dept. Anat. and Neurobiol., Univ. Kentucky Med. Ctr.; [2]Sch. Nat. Sci., Hampshire College; [3]Prog. in Neuronal Growth & Dev., Dept. Anat. & Neurobiol., Colorado State Univ.

Growth factors (GFs) and neurotransmitters are believed to play important signaling roles in the development and maintenance of functional neuroarchitecture, and imbalances in these signals may lead to neurodegeneration in aging and disease. The actions of GFs and neurotransmitters have been studied separately in the past, but it is likely that neurons are exposed to these agents simultaneously *in situ*. We therefore tested the hypothesis that these two classes of signals might interact. As expected from previous studies, fibroblast growth factor (FGF) promoted cell survival and neurite outgrowth, while glutamate reduced survival and dendritic outgrowth in cultured rat hippocampal pyramidal neurons. FGF reduced glutamate-induced neurodegeneration; the threshold for glutamate neurotoxicity was elevated. The large and sustained rises in intracellular calcium normally caused by glutamate were reduced by FGF. FGF-maintained neurons were better able to reduce A23187-induced rises in intracellular Ca^{2+} levels suggesting that FGF improved the efficacy of Ca^{2+} extrusion systems. FGF's ability to reduce the Ca^{2+}-elevating and degenerative actions of glutamate required prolonged exposure to FGF and were blocked by actinomycin D and cycloheximide indicating that protein synthesis was involved in FGF's actions. Taken together, these data demonstrate that excitatory amino acids (EAAs) and GFs can have opposing actions on neurite outgrowth and cell survival. We suggest that interactions between EAAs and growth factors may regulate the development and plasticity of neural circuitry, and that imbalances in these systems may lead to pathological neurodegeneration. (Supported by the French Foundation for Alzheimer's disease).

499.8

DOPAMINERGIC CELL NUMBER IS SELECTIVELY INCREASED BY SUBSTANTIA NIGRA SUPPORT CELLS *IN VITRO*. E.K. O'Malley, I.B. Black, and C.F. Dreyfus. Div. Devel. Neurol., Cornell Univ. Med. Coll., N.Y., N.Y. 10021

We have previously found that neuron-support cell interactions selectively increase dopaminergic (DA) cell number and function in cultured substantia nigra (SN). We now present evidence that these actions are mediated specifically by the SN support cell population.

Support cell monolayers were established in serum-containing medium, rinsed thoroughly and maintained in serum-free medium. Dissociated neurons from embryonic day 16 rat SN's were plated alone (control), or on support cell beds. Tyrosine hydroxylase (TH), the rate-limiting enzyme in dopamine biosynthesis, was used to monitor SN development.

To examine the specificity of support cell actions, monolayers were derived from local (SN), target (striatum,) or non-target (occipital cortex,) brain regions. TH catalytic activity induced by SN support cells was significantly higher than all other groups, suggesting that the local environment specifically fosters DA cell development. To determine whether this effect on enzyme activity reflected alteration in cell number, neurons were immunostained for TH or the neuronal marker, neuron specific enolase (NSE). SN support cells elicited a selective increase in the DA subpopulation, since TH cell number increased, while NSE cell number did not differ from control. In contrast, target and non-target support cells increased both TH and NSE cell number. These data suggest that specific local support cell factors increase DA neuron number in the SN. (supported by NIH grant HD 23315.)

499.9

REGULATION OF CELL ADHESION MOLECULE (CAM) LEVELS ON CULTURED RAT SCHWANN CELLS BY EPIDERMAL GROWTH FACTOR (EGF) A. Acheson, P. Barker*, J.G. Toma*, T.C. Mathew*, F. Miller and R.A. Murphy. Dept. of Anatomy and Cell Biology, Univ. of Alberta, Edmonton, Alberta CANADA T6G 2H7

Recent interest in Schwann cells has focussed on their potential role in peripheral nerve regeneration, in particular on trophic interactions with regenerating neurons. Schwann cells have NGF receptors, and levels of the L1 CAM are regulated in vitro by NGF (Seilheimer & Schachner, EMBO J. 6: 1611, 1987). Using Schwann cells purified from neonatal rat sciatic nerve, our Northern blot analyses show that Schwann cells express EGF receptor (EGF-R) mRNA. Treatment of Schwann cells with EGF produces a dose- and time-dependent increase in L1 protein levels, as determined by semi-quantitative immunoblots. Levels of the neural cell adhesion molecule (NCAM) also increase, although to a lesser extent. Immunostaining of frozen sections of sciatic nerve also reveals a time-dependent increase in both L1 and NCAM immunoreactivity in the proximal stump after nerve transection.

These data indicate that Schwann cells express functional EGF-R in vitro and that EGF treatment alters levels of CAMs. Taken together with the observed increases in CAM immunoreactivity after sciatic nerve transection, our results suggest that EGF, or another trophic factor which binds to EGF-R, could be involved in altering Schwann cell CAMs in vivo, thus facilitating nerve regeneration.

499.10

MORPHOLOGICAL AND BIOCHEMICAL ALTERATIONS IN DEVELOPING SYMPATHETIC NEURONS PROVIDED WITH EXCESS TARGET SPACE. A.J. Smolen, L. Cosio*, F. Scholl*, P. Beaston-Wimmer*, and T. Cianci*. Dept. of Anatomy, Medical College of Pa., Philadelphia, PA 19129.

The pineal gland receives its innervation from sympathetic neurons located bilaterally in the superior cervical ganglion (SCG). In the present study, one SCG was surgically removed from newborn rats, and the animals were permitted to survive to adulthood.

In the pineal glands of operated animals, both content and turnover of the sympathetic neurotransmitter, norepinephrine (NE), were normal. Therefore, in the absence of competing innervation from the contralateral side, SCG neurons are capable of increasing their functional innervation to the pineal.

The cell bodies and the dendritic trees of these sympathetic neurons which had formed increased innervation in the pineal were examined by injecting the pineal with a conjugate of horseradish peroxidase and cholera toxin (HRP-CT). The cell bodies of the HRP-CT labelled neurons were larger than in controls, but the dendritic trees of these neurons were not enlarged. The number of branches and total dendritic length were decreased, while dendritic diameter was increased. Overall, the total volume of the dendritic trees was normal, and the total neuronal volume was increased.

These results indicate that an expansion of a neuron's axonal field is not necessarily accompanied by a concomitant expansion of its dendritic field. The increase in volume of the cell body that we observed may be due to a need by these neurons for enhanced production of neurotransmitter. (Supported by NS15952 and NS21822)

499.11

INCREASED FORMATION OF ENDOTHELIN LIKE IMMUNOREACTIVITY IN ACTIVATED ASTROGLIA AND ITS RELATION TO 125I-ENDOTHELIN-1 BINDING SITES IN THE RAT BRAIN. K. Fuxe, A. Cintra*, E. Änggård*, B. Tinner*, W. Staines, L.F. Agnati*, S. Galton* & J.R. Vane. Dept of Histol & Neurobiol, Karolinska Inst, Stockholm, Sweden; William Harvey Res Inst, St Bartholomew's Hospital Med College, London, U.K.; Dept of Human Physiol, Univ of Modena, Modena, Italy; Dept of Anatomy, Univ of Ottawa, Ottawa, Ontario, Canada.

The possible existence of endothelin mechanisms has been analyzed in the male rat brain by means of the indirect immunofluorescence technique using a rabbit antiserum (Peptide Institure, Osaka, Japan) against endothelin-1 (ET-1) and quantitative receptor autoradiography, using as a radioligand 125I ET-1. Excitotoxic lesions were produced in the hippocampal formation by means of local injections of ibotenic acid (5 µg/0.5 µl, 7 days before killing). These hippocampal lesions markedly increased ET-like IR in astroglial cells with processes surrounding small blood vessels, particularly within the stratum lacunosum moleculare. These cells costore glial fibrillary acidic protein (GFAP) as evidenced by using a mouse monoclonal antibody against GFAP (Boehringer-Mannheim, FRG). A match was observed between ET IR in astroglial cells and ET-1 binding sites within the stratum lacunosum moleculare. Thus, ET-like peptides may participate in neuronal-glial interaction and in control of local cerebral blood flow and may represent glial hormones for the trophic regulation of the CNS.

PROCESS OUTGROWTH, GROWTH CONES AND GUIDANCE MECHANISMS X

500.1

Saltatory extension of pioneer growth cones along single filopodia in vivo. **T. P. O'Connor, J. S. Duerr* and D. Bentley. Department of Molecular and Cell Biology, University of California, Berkeley, CA 94720.**

Models of growth cone migration include axon advancement (1) along a broad front, (2) by filling in between groups of filopodia, and (3) by selective conversion of single filopodia. Using computer-enhanced image intensification, we examined the migration of DiI labeled growth cones of grasshopper peripheral pioneer neurons in vivo. Starting at the 30% stage of embryogenesis, these growth cones migrate along a stereotyped route through limb buds while responding to a sequence of described guidance cues. We observed that these growth cones regularly advance in vivo by conversion of a single filopodium to a growth cone branch, followed by maturation of the branch into an axon. Such an event results in a stepwise or saltatory advance of the growth cone to the point formerly occupied by the tip of the filopodium. This method of extension can occur while the growth cone is advancing directly toward a pre-axonogensis (guidepost) neuron (Tr1), and also can mediate the steering event which results in the growth cone turning toward the Cx1 guidepost neurons. Advancement by conversion of single filopodia suggests the presence of powerful amplification mechanisms for reorienting the entire growth cone on the basis of single filopodial contacts.

500.2

GUIDEPOST CELLS ACT AS CALCIUM SINKS FOR PIONEER GROWTH CONES *IN VIVO*. P.B. Guthrie, S.B. Kater, and D. Bentley. Program in Neuronal Growth & Development and Dept. of Anatomy & Neurobiology, Colorado State University, Ft Collins, CO 80523; Dept. of Molecular & Cell Biology, University of California, Berkeley, CA 94720.

Growth cones of pioneer (Ti1) neurons in embryonic grasshopper limb buds arise at the limb tip. As they migrate along a stereotyped route to the CNS, they interact with a variety of epithelial and neuronal substrates. The highest affinity substrate appears to be a series of guidepost neurons. Since calcium has been implicated as a primary regulator of growth cone behavior in both vertebrate and invertebrate neurons, we examined calcium regulation in migrating pioneer growth cones *in vivo*. We report here that guidepost cells may regulate calcium levels in pioneer growth cones.

One of the pair of pioneer neurons (stages 31%-35%) was injected with fura-2 from a microelectrode. At all stages examined, the two pioneer neurons were dye-coupled to each other; however, dye-coupling to other cells was stage dependent. Pioneer neurons became dye-coupled to the first (Fe1) guidepost cell at about the 32.5% stage, and to the second (Tr1) guidepost at about the 33.5% stage. Although segment boundary epithelial cells have relatively high affinity for pioneer growth cones, dye-coupling to these cells was never observed.

Intracellular calcium levels were typically higher in pioneer growth cones than in cell bodies. Guidepost cells always had lower calcium concentrations than pioneer neurons. In addition, pioneer axons had lower calcium levels where they crossed guideposts, suggesting that the guideposts may act as calcium sinks.

To test this possibility, we raised calcium levels of fura-2 filled cells by local, brief UV irradiation. Irradiation of pioneer axons proximal to the first guidepost elevated calcium levels locally; the region crossing the guidepost remained unchanged. Irradiation of the axons between guideposts elevated calcium in that region, but had minimal effects beyond the guideposts. Direct irradiation of either guidepost elevated calcium levels both of the guidepost, and of adjacent regions of the pioneer axons. These data suggest that guidepost cells act as calcium sinks for the pioneer neurons.

500.3

CELL-CELL INTERACTIONS DURING THE FORMATION OF A COMMISSURAL PATHWAY IN THE EMBRYONIC GRASSHOPPER. Paul Z. Myers* and Michael J. Bastiani (SPON: J. Petajan). Dept. of Biology, Univ. of Utah, Salt Lake City, UT 84112.

We want to understand why the growth cones of commissural neurons bypass an ipsilateral target to select a presumably equivalent, but more distant, contralateral target. One hypothesis that we are pursuing is that commissural neurons have their targets re-specified during axonal outgrowth, perhaps after reaching the midline, by specific cellular interactions.

In the grasshopper, the posterior commissure is pioncered by an identifiable neuron called Q1. The Q1 growth cone grows over the ipsilateral MP1/dMP2 axon, which pioneers the longitudinal fascicle, and crosses the midline to turn and grow caudally along the contralateral MP1/dMP2 axon. During axonal outgrowth, Q1's growth cone contacts and dye-couples with only a small number of neurons. Ipsilaterally, Q1 contacts and couples to the MP1/dMP2 neuron and the anterior and posterior corner cells, which also extend growth cones along MP1/dMP2. At the midline, the Q1 growth cone contacts its contralateral homolog and a midline neuron, MP4, and also becomes dye-coupled to the contralateral MP1/dMP2 and corner cells.

We are ablating the cells that the Q1 growth cone contacts to identify those neurons that are required for the formation of a normal commissure. Preliminary results suggest that the Q1 growth cone requires the presence of its contralateral homolog in order to follow its normal course across the midline.

(Supported by NIH NS25387, the McKnight Foundation, and the National Cancer Institute)

500.4

PERTURBATION OF AXON GROWTH IN THE GRASSHOPPER LIMB BUD BY CHROMOPHORE ASSISTED LASER INACTIVATION. H. Keshishian & D. G. Jay*. Dept. of Biology, Yale Univ., New Haven, CT & Dept. of Cellular and Developmental Biol., Harvard Univ., Cambridge, MA.

Chromophore assisted laser inactivation (CALI) (Jay, D.G. PNAS 85:5454-58, 1988) was used to study axonal growth and guidance in the grasshopper limb bud. This procedure inactivates single protein functions with a dye labeled antibody subjected to nanosecond pulses of laser light of a wavelength absorbed by the dye but not by cellular components. The dye targets laser energy to denature bound proteins, leaving others relatively unaffected. The Ti1 pioneer neurons are a cell pair whose tightly fasciculated axons project stereotypically to the CNS. In insects, HRP binds specifically to a sugar moiety of neuronal membrane glycoproteins including the fasciclins. Dye labeled anti-HRP was injected into 30% grasshopper embryos that were subjected to laser irradiation to inactivate these proteins. The progress of axonal growth was visualized after 24 hours of further incubation. We found a three fold increase of defasciculation relative to nonirradiated controls(n=80). Other controls included nonirradiated and irradiated embryos injected with either unlabeled anti-HRP, or dye labeled BSA. Defasciculation occurred in about 1/3 of the CALI treated limb buds. Often axons separated for up to 100 micrometers before rejoining as they progressed proximally. We also found examples of aberrant axonal growth and guidance. These experiments suggest a role for one or more of the anti-HRP cross reactive antigens in axonal adhesion. This demonstrates the use of CALI in studying molecular events in neurodevelopment.

500.5

SOMA POSITION DETERMINES IDENTITY OF PRIMARY MOTONEURONS IN DEVELOPING ZEBRAFISH EMBRYOS. J.S. Eisen. Institute of Neuroscience, University of Oregon, Eugene OR 97403.

The axial muscles of the zebrafish are innervated by a segmentally repeated set of three primary motoneurons. The two features that allow each primary motoneuron to be identified, its soma position and the region of muscle it innervates, are always correlated. To learn whether soma position specifies the region of muscle a motoneuron will innervate, I transplanted identified motoneurons between embryos.

Transplanted cells were placed in their normal position or shifted to the position of another motoneuron. Young motoneurons without axons developed normal arbors when transplanted to embryos of the same stage. A motoneuron whose soma position was shifted developed an arbor appropriate for its new soma position, suggesting that its identity was not fixed prior to axogenesis. Older motoneurons with axons also formed arbors appropriate for their new soma positions, when transplanted into hosts 1-2 hrs younger. This result suggests that motoneuronal identity was not fixed even after axogenesis. However, motoneurons with axons failed to form arbors when transplanted into hosts of the same stage as the donor embryos. Young motoneurons transplanted into embryos 1-2 hrs older also failed to form arbors.

These results suggest that soma position determines motoneuronal identity by specifying which region of muscle a motoneuron will innervate. While motoneuronal identity appears plastic even after axogenesis, age-related environmental features may regulate the expression of that identity by limiting the ability of a motoneuron to arborize. Supported by NS23915 and BNS8553146.

500.6

EXTENSION OF MOTONEURONAL GROWTH CONES IS DISRUPTED BY THE ABSENCE OF PRE-EXISTING AXONAL PATHWAYS. S.H. Pike*, E.F. Brandenburg*, and J.S. Eisen. (SPON: C. Gatchalian). Institute of Neuroscience, University of Oregon, Eugene, OR 97403.

The growth cones of zebrafish secondary motoneurons follow pathways established by the axons of pioneering primary motoneurons. This observation suggests that growth cones of secondary motoneurons may require the axons of primary motoneurons for normal extension. To test this model, we ablated primary motoneurons prior to axogenesis by laser-irradiation, and examined subsequent axonal outgrowth of the secondary motoneurons. We found that the axons of secondary motoneurons had not extended along pathways lacking primary motoneuron axons at stages when secondary motoneurons in control segments had grown to the distal limits of these pathways. Thus, secondary motoneuron outgrowth is either delayed or arrested in the absence of primary motoneuron axons. These results support the hypothesis that the axons of primary motoneurons are important for normal axonal extension of secondary motoneurons. Supported by NS23915.

500.7

PRIMARY MOTONEURONS INSTRUCT MUSCLE ACETYLCHOLINE RECEPTOR PLACEMENT, BUT RECEPTORS DON'T INSTRUCT MOTONEURONAL SYNAPSE PLACEMENT, IN LIVE ZEBRAFISH. D.W. Liu & M. Westerfield, Institute of Neuroscience, University of Oregon, Eugene, OR, USA.

Inductive interactions between nerve and muscle are known to be important for the development of each cell type. We have investigated the role of these interactions in the specification of synaptic connections by watching directly the development of labelled acetylcholine (ACh) receptor clusters on muscle fibers during innervation by labelled primary motoneurons in live zebrafish embryos, and by selectively eliminating either the neurons or the receptors.

We found that prior to contact by motoneuronal growth cones, the muscles lacked clustered receptors. Within minutes after a growth cone first contacted a muscle fiber, ACh receptors clustered at the site of contact and functional neuromuscular transmission occurred. In mutant zebrafish lacking ACh receptors motoneuronal development was normal; neuromuscular junctions were found on appropriate muscle fibers. However ablation of primary motoneurons prior to axogenesis had a profound effect on the expression of muscle receptors; causing them to cluster ectopically and much later than normal.

We conclude that, in the zebrafish embryo, the influence of the motoneuron is required for appropriate clustering of muscle ACh receptors, whereas ACh receptor-mediated interactions are not necessary for the establishment of specific synaptic connections by the primary motoneurons. Supported by NS21132 & GM07257 & HD22486

500.8

LOSS OF A SURFACE ANTIGEN FROM CORTICAL NEURONS IN VITRO FOLLOWING TREATMENT WITH THE CNS-SPECIFIC ANTIBODY 2A1. N.L. Baumrind, M.P. Sheetz*, D.B. Wayne*, and A.L. Pearlman. Depts. of Cell Biology and Neurology, Washington Univ. Medical School, St. Louis, MO 63110.

The 2A1 antigen is transiently expressed in the murine central nervous system during development. Immunoreactivity is present in areas that contain post-mitotic neurons and their processes, and is excluded from proliferative zones. In tissue culture the antigen is on the surface of neurons but not on astrocytes or fibroblasts (Baumrind et al., Neurosci Abst., 1985). Immunoreactivity is not present in the peripheral nervous system or non-neural tissues. Immunolabeling of embryonic cortical neurons in vitro is prominent along filopodia and the perimeter of growth cones, and is markedly enhanced at points of contact between neurites. Treatment of cultures with 2A1 (divalent antibody) leads to selective loss of the 2A1 antigen from neuronal surfaces; NCAM and A2B5 continue to be distributed normally. Loss of 2A1 antigen from neuronal surfaces; NCAM and A2B5 continue to be distributed normally. Loss of 2A1 antigen from the surface of treated neurons is accompanied by neurite retraction and eventual death of neurons, while non-neuronal cells are unaffected. As 2A1 immunoreactivity is lost from neuronal surfaces it appears as aggregates within the cell body. Video enhanced DIC observation of gold particles coated with 2A1 demonstrates endocytosis of particles along processes and growth cones.

500.9

ACTIVE OUTWARD MOVEMENT OF MATERIAL WITHIN WITHIN ACTIN-RICH REGIONS OF NEURONAL GROWTH CONES. M.P. Sheetz*, N.L. Baumrind, D.B. Wayne and A.L. Pearlman (SPON: D. Parkinson). Depts. of Cell Biol. & Neurol., Wash. Univ. Med. School, St. Louis, MO 63110.

A very prominent activity in growth cones is the backward or retrograde movement of waves in the actin matrix which corresponds with backward movements of actin. Video-enhanced DIC shows retrograde movement even within filopodia of cortical neuronal growth cones (embroynic mouse, El3). Surprisingly, we also observed rapid forward movement within filopodia (see Wayne et al., this issue). Within many filopodia, swellings move toward the front of filopodia at 0.5-2 microns/sec. Movements are often discontinuous and packets can reverse direction. Transport was not observed in all filopodia and could be an unusual phenomenon; however, in the best optical situations frequent forward movements of smaller packets were seen indicating the presence of more extensive movements. No microtubules were seen in filopodia stained within antitubulin antibody. Active forward movements suggest that forward propulsion could drive process extension. Similarly, retrograde movements could be fueled by packets transported forward. An active transport model for processes extension is proposed.

500.10

POSITIONAL INFORMATION IN BOTH THE EYEBUD AND THE TECTUM GUIDE THE FORMATION OF THE RETINOTECTAL MAP IN *XENOPUS*. S.E. Fraser. Dept. of Physiology & Biophysics, University of California, Irvine, CA 92717

This study examines the nature and presence of positional cues in the formation of the topographic projection from the retina to the optic tectum. *Xenopus* eyebud fragments labeled with lysinated fluorescein dextran (LFD) or lysinated rhodamine dextran (LRD) were grafted either to equivalent locations from which they were removed (homotopic) or to non-equivalent positions (heterotopic) in host eyebuds. Some animals received grafts containing only a few, and occasionally one, dye labeled cells. Because the dyes were transported along the optic axons, it was possible to visualize the projections of individual optic nerve fibers including their growth cones in living animals using computer enhanced low light level epifluorescence microscopy.

For homotopic grafts, the results were equivalent to those found with previous grafting experiments using large eyebud fragments: dorsal, ventral, anterior, and posterior retinal grafts projected to ventral, dorsal, posterior and anterior tectum, respectively. In contrast, heterotopically transplanted eyebud fragments, including individual cells, projected to the tectum according to their site of origin in the donor, independent of their position in the host. Subsequent observation of the labeled optic nerve fibers demonstrated that this pattern remained stable for at least two weeks.

To determine if cues present on the optic tectum can guide optic nerve fibers, the labeled optic nerve fibers were allowed to innervate the tectum in the absence of other optic nerve fibers, the other potential positional cue. Host eyebuds were ablated prior to outgrowth of optic nerve fibers, and two eyebud fragments (one labeled with LFD and the other labeled with LRD) from either nasal and temporal or dorsal and ventral retina were grafted onto the same optic stalk of the unlabeled host. Even in the absence of other optic nerve fibers, these grafted eyebud cells were able to find their correct target sites on the tectum. This indicates that positional cues not only on the eyebud but also on the tectum guide the formation of the topographic retinotectal map.

500.11

CEREBELLAR AFFERENT INTERACTIONS WITH TARGET AND NON-TARGET CELLS IN VITRO. D.H. Baird, Y. You*, L.B. Friedman*, M.E. Hatten and C.A. Mason. Dept. of Pathology, College of Physicians and Surgeons of Columbia University, P&S, New York, N.Y, 10032.

Before afferents interact with target cells, axonal growth cones extend through tracts and the target region, encountering other neurons and non-neuronal cells throughout this trajectory. To understand how neurite extension and growth cone behavior is influenced by both target and non-target cells, we examined neurite extension and growth cone interactions in our model culture system. Brainstem sources of cerebellar afferents (pontine nuclei for mossy fibers) were cocultured with dissociated, purified populations of target (granule) neurons, non-target neurons and astroglia. Explant neurites from mouse were identified on monolayers of cells from rat with the monoclonal antibody M6 (gift of K. Lagenaur), which stains mouse neurons exclusively. Explant neurites fasciculate on the polylysine substrate in the absence of cells, or when grown adjacent to cells. Fasciculation is much reduced when neurites associate with target or non-target cells. While neurite outgrowth is abundant on cellular monolayers, neurite length is diminished on target neurons as compared to non-target cells from cerebellum or hippocampus. When analyzed with video-enhanced DIC microscopy, behavior of individual afferent growth cones with various cell types correlates with the above growth patterns: rapid elongation and fasciculation when pontine neurites meet each other; temporary cessation of elongation and maintenance of cell-cell contacts when pontine growth cones meet targets. These results suggest that target neurons temporarily arrest afferent neurite extension, while contact with non-target cells permits elongation.

500.12

REGIONAL SPECIFICITY OF GLIAL-GUIDED NEURONAL MIGRATION: CEREBELLAR GRANULE NEURONS MIGRATE ON HIPPOCAMPAL ASTROGLIAL FIBERS *IN VITRO*. U.E. Gasser and M.E. Hatten, Department of Pathology, College of Physicians and Surgeons of Columbia University, New York, NY 10032.

In most cortical regions of the developing mammalian brain, radially oriented astroglial fibers provide the primary pathway for neuronal migration. To test the regional specificity of glial-guided migration, we "mixed and matched" neurons and astroglia purified from late embryonic and early postnatal rat cerebellum and hippocampus *in vitro*, and analyzed glial-guided neuronal migration with video-enhanced differential interference contrast microscopy (Edmondson, J.C. and M.E. Hatten, J. Neurosci.7:1928,1987). In both homotypic and heterotypic co-cultures, a stereotyped neuron-glia apposition, neuronal cytology, mode of movement and speed of migration (25-60 um/h) along glial fibers was seen. These experiments suggest that the mechanism of glial guidance of neuronal migration is conserved across brain regions and that astroglial fibers provide a "generic" pathway for neuronal migration in the developing brain. The regulation of the timing, distance and termination of migration, seen to vary so greatly among types of neurons in various cortical regions is therefore likely to be mediated by neuron-neuron interactions rather than neuron-glial interactions.

INVERTEBRATE LEARNING AND BEHAVIOR III

501.1

OPERANT CONDITIONING CAN BE SIMULATED BY SMALL NETWORKS OF NEURON-LIKE ADAPTIVE ELEMENTS. D.A. Baxter, J.L. Raymond, D.V. Buonomano and J.H. Byrne. Department of Neurobiology and Anatomy, University of Texas Medical School, Houston, TX 77225.

Activity-dependent neuromodulation has been proposed as a cellular mechanism for classical conditioning in *Aplysia*. Previously, we developed a mathematical model of an *Aplysia* sensory neuron that reflects the subcellular processes underlying this form of associative plasticity. This single-cell model can simulate features of non-associative learning and classical conditioning (Gingrich and Byrne 1985, 1987). In addition, mathematical models of small networks containing adaptive elements with this activity-dependent neuromodulation 'learning rule' can simulate some higher-order features of classical conditioning (Gluck and Thompson 1987; Byrne et al. 1988; Hawkins 1989). In the present study, we test the hypothesis that this learning rule can also simulate features of operant conditioning.

We use a network that contains seven elements, two of which are adaptive elements with the associative learning rule. A central pattern generator (CPG) consisting of two spontaneously active and mutually inhibitory neurons drives the network between two possible output states, each state being determined by activity in one of the CPG neurons. Each of the CPG neurons drives an adaptive element, which in turn drives a motor neuron to produce one of the output states. Feedback from each motor neuron to that component of the CPG which drives it, regulates the duration of that component's burst. Reinforcement is provided by a modulatory neuron that synapses onto both adaptive elements.

We simulated operant conditioning by activating the modulatory neuron whenever one selected output state occurred. This led to changes in the relative amount of time the network spent in each of the output states. These changes are analogous to changes seen in studies of operant conditioning. Thus, our simulations illustrate that as an associative learning rule, activity-dependent neuromodulation can simulate features of a number of different types of learning, including operant conditioning. Supported by grant AFOSR 87-0274.

501.2

ACTIVITY-DEPENDENT BLOCK OF CENTRAL SENSORY CONDUCTION DURING INHIBITION OF TAIL WITHDRAWAL REFLEX IN APLYSIA. A.L.Clatworthy* and E.T. Walters. Dept. of Physiology & Cell Biology, Univ. Texas Med.Sch, Houston, TX 77225.

Presynaptic inhibition of mechanosensory neurons may be a mechanism of tail shock-induced inhibition of siphon withdrawal (Mackey et al. 1987). Knowing that some sensory plasticity in Aplysia involves cell-wide modulation of signaling strength (Billy & Walters 1989), we predicted that behavioral inhibition might involve central conduction block in sensory axons. A train of test stimuli to nerve p9 was used to activate axons of tail sensory neurons and elicit tail contractions. Intense shock of another nerve (p8) or tail pinch significantly inhibited contractions evoked by p9 test stimuli for at least 10 min. Stimulation of p8 decreased the number of p9-elicited spikes recorded in tail sensory neuron somata in 10 of 20 preparations. Tail pinch outside the sensory neuron receptive field had little effect on spike conduction in p9 tests. However, tail pinch inside the receptive field (activating the cell) blocked conduction of all p9-evoked spikes 10 sec after the pinch in 7 of 8 preparations. Spike conduction showed partial recovery 5 min after the pinch. Conduction block thus appears to be a novel, potent mechanism for behavioral plasticity. Alterations underlying the block are likely to have interesting relationships to presynaptic inhibition.

501.3

CONDITIONING OF NOVEL SIPHON RESPONSES IN APLYSIA SUGGESTS A SIMPLE S-R ASSOCIATION MODEL. E.T. Walters and D.A. Fenyes*. Dept. of Physiology & Cell Biology, Univ. Texas Medical School, Houston, TX 77225.

Previous studies of siphon conditioning in Aplysia described quantitative changes in preexisting responses (alpha Rs) but no novel CRs. We have used a reduced preparation to test the possibility that a CS to the mantle, which normally causes constricting siphon Rs, will cause flaring siphon Rs resembling the UR after pairing the CS with a US to nerves from the tail. Animals (n=14) receiving 5 CS-US pairings showed a significantly greater incidence of flaring CRs (monitored with a photocell and automated program) than animals receiving unpaired CS and US presentations (n=15), the CS alone (n=14), or US alone (n=14). The unpaired and US-alone groups also showed some pseudoconditioning of flaring Rs, replicating previous observations (Erickson & Walters 1988). These results and known cellular plasticity in involved circuits (Hawkins et al. 1983; Frost et al. 1988) suggested an associative model based upon concatenation of cell-wide (rather than synapse-specific) mechanisms of CS-specific sensory facilitation and UR-specific motor facilitation. Computer simulations confirm that this simple model can produce effective associations in generalized S-R networks, and that UR-like CRs are more likely when the alpha R and UR are mutually incompatible actions.

501.4

CLASSICAL CONDITIONING OF *APLYSIA* SIPHON-WITHDRAWAL INVOLVES THE DEVELOPMENT OF A NEW RESPONSE TO THE CS. R.D. Hawkins, N. Lalevic*, G.A. Clark, and E.R. Kandel. Ctr. Neurobiol. & Behav., Columbia Univ., HHMI, & NYSPI, New York, New York 10032.

The gill- and siphon-withdrawal reflex of *Aplysia* undergoes classical conditioning of its amplitude and duration when siphon stimulation (the CS) is paired with tail or mantle shock (the US). This conditioning of a pre-existing response exhibits both temporal and stimulus specificities which can be accounted for by activity-dependent presynaptic facilitation of the siphon sensory neurons. To test whether conditioning of the reflex also exhibits response specificity (development of a new response to the CS which resembles the response to the US), we measured the direction of siphon withdrawal in response to siphon stimulation (the CS) with tail or mantle shock as the US. The naive response to siphon stimulation is straight contraction, the response to tail shock is backward bending, and the response to mantle shock is forward bending. In the first experiment we trained different animals with the two USs, and in a second experiment we trained each animal with both USs, one of which was paired with the CS. There was a significant, pairing-specific tendency for the direction of the response to the CS to resemble the response to the US following training in both experiments. This feature of the conditioning might be accounted for by an elaboration of activity-dependent facilitation.

501.5

TRIAL-TO-TRIAL VARIABILITY IN THE NEURONAL RESPONSE TO SIPHON TOUCH IN THE APLYSIA ABDOMINAL GANGLION. J.-Y. Wu, C. X. Falk*, H.-P. Hopp, and L.B. Cohen. Dept. of Physiology, Yale Univ. Sch. of Med., New Haven CT, 06510.

Optical recordings allow one to monitor the activity of a substantial fraction (~50%) of the neurons in the Aplysia abdominal ganglion. While previous results suggested that as many as 300 neurons might be involved in the gill-withdrawal reflex, we had only sketchy information about how reproducible the neuronal response was from trial to trial in one preparation. When the same stimulus was given to the siphon in four trials both the number of active neurons and the total number of spikes were very similar from trial to trial. However only a fraction of the neurons were the same from trial to trial. In five preparations, this fraction ranged from 41% to 79%. Surprisingly, in experiments where the stimulus strength was varied (between 0.5 and 4.0g) there was a similar constancy of number of active neurons across trials. However, in these experiments, the number of actions potentials per neuron usually increased with increasing stimuli.

501.6

BLOCKING GILL AND SIPHON MOVEMENTS HAS LITTLE OR NO EFFECT ON THE NEURONAL RESPONSE TO SIPHON TOUCH IN THE APLYSIA ABDOMINAL GANGLION. C.X. Falk*, H.-P. Hopp, J.-Y. Wu, and L.B. Cohen. Dept. of Physiology, Yale Univ. Sch. of Med., New Haven CT, 06510.

Previous results from optical recordings suggested that as many as 300 neurons in the abdominal ganglion might be involved in the gill-withdrawal reflex. We began efforts to determine if the reflex could be elicited in conditions where a smaller number of neurons might be involved. We compared the number of active neurons in the control situation with sea water bathing the siphon, ganglion, and gill, and the blocked situation where the siphon and gill were bathed in a low-calcium high-magnesium sea water to block contraction and possible recurrent sensory feedback. In all the experiments where measurements in blocker were bracketed by measurements in sea water the number of active neurons was apparently unaffected. In addition the timing of their activity was also similar. In one experiment, the presence of blocker did reduce the number of active neurons by about 30% but a recovery measurement in sea water was not done. Thus recurrent activity from siphon and gill contractions did not have large effects on the neuron activity in the ganglion.

501.7

ANALYSIS OF A NEWLY DESCRIBED CELLULAR PROCESS CONTRIBUTING TO FACILITATION AT DEPRESSED SENSORY NEURON SYNAPSES IN *APLYSIA*. M. Klein[1]*, Q. Braha[1], N. Dale[1], E.R. Kandel[1], C.A. Hansen[2] and T.W. Abrams[3] (SPON: B. Chance). [1]Center for Neurobiol. & Behavior and HHMI, Columbia Univ., New York, NY, and [2]Dept. of Biochemistry & Biophysics and [3]Dept. of Biology & Instit. of Neurological Sci., Univ. of Penn., Phila., PA.

Facilitation of sensory neuron (SN) to motoneuron synapses in *Aplysia* results, at least in part, from a cAMP-dependent prolongation of the presynaptic action potential. Hochner et al. (1986) demonstrated that an additional process is required for facilitation of depressed synapses. We have sought to determine whether cAMP may be involved in the second process responsible for facilitation of depressed synapses. We investigated the ability of cAMP to facilitate depressed synapses using photolysable "caged" cAMP (dimethoxynitrobenzyl cAMP) in experiments on synapses between single SNs and LFS motoneurons in culture. Light-induced release of cAMP caused facilitation of depressed synapses, as well as of non-depressed synapses, suggesting that a cAMP-induced process may participate in facilitation of depressed synapses. In a second series of experiments, we used H7, a kinase inhibitor that was ineffective in blocking effects of cAMP on SN excitability, to explore the possible involvement of other kinases. H7 greatly reduced the facilitation of depressed synapses produced either by 5-HT or by photolysis of caged "cAMP". These results suggest that a second kinase or another H7 sensitive process may act downstream from the cAMP-dependent kinase in the facilitation of depressed synapses.

501.8

ROLE OF ADENYLATE CYCLASE IN SEVERAL FORMS OF SYNAPTIC FACILITATION IN *APLYSIA* SENSORY NEURONS. B.A. Goldsmith* and T.W. Abrams Dept. of Biology & Institute of Neurological Sciences, Univ. of Pennsylvania, Phila., PA 19104.

The synaptic connections from *Aplysia* sensory neurons (SNs) to motoneurons undergo at least three forms of heterosynaptic facilitation in response to the facilitatory transmitter 5-HT. Facilitation of non-depressed SN synapses results, at least in part, from the cAMP-dependent prolongation of the presynaptic action potential. Facilitation of depressed SN synapses involves an additional process, possibly mediated by another second messenger system (Hochner et al., 1986). Activity-dependent synaptic facilitation of these same SN synapses involves yet another intracellular signal, probably calcium influx, which is a consequence of the SNs' own activity. To explore the role of adenylate cyclase in removal of synaptic depression and in activity-dependent facilitation, we have used an inhibitor of adenylate cyclase, tetrahydrofuryladenine (THFA). When tested *in vitro*, 5 mM THFA blocked more than 80% of adenylate cyclase activity, but had no effect on cAMP-dependent kinase activity. Applied extracellularly, THFA substantially reduced the cAMP-dependent broadening of the SN action potential produced by 5–HT. THFA also greatly inhibited the facilitation by 5-HT of depressed SN synapses in the abdominal ganglion. Both effects of THFA were reversible. These results suggest that adenylate cyclase plays an important role in removal of synaptic depression as it does in facilitation of non-depressed SN synapses. We are presently using THFA to investigate the contribution of adenylate cyclase to activity-dependent facilitation.

501.9

DEVELOPMENT OF NEUROMODULATION IN THE PLEURAL SENSORY NEURONS OF *APLYSIA*. E.A. Marcus and T.J. Carew. Depts. of Biology and Psychology, Yale University, New Haven, CT 06520

Sensitization in *Aplysia* does not emerge until relatively late in juvenile development (Late Stage 12). In adult *Aplysia*, sensitization is known to be mediated at least in part by 5HT-induced modulation of identified sensory neurons (SNs). Taken together, these data suggest the possibility that some component(s) of the biophysical and/or molecular mechanisms activated by 5HT in the adult may be lacking in earlier juvenile stages. We have begun to address this question by examining the effects of 5HT on the pleural SNs at different stages of juvenile development.

One of the known effects of 5HT in adult animals is to produce an increase in excitability (anti-accomodation) which is reflected in an increase in the number of action potentials produced by a constant current injection in the soma. We have found that in Late Stage 12, as in the adult, 5HT (50 μM) has a significant anti-accomodation effect on SN firing ($\bar{x}$=229% of baseline, p<.006, N=9). This effect is readily reversible and cannot be accounted for by effects of 5HT on membrane potential, since it is not mimicked by tonic depolarization or hyperpolarization of the SN. Preliminary evidence suggests that other facilitatory effects of 5HT (spike broadening) are also present at this stage. Thus it appears that at least some of the ionic currents known to be modulated by 5HT are present in the SNs at this stage of development. It will now be interesting to examine these effects in progressively earlier juvenile stages.

We are also currently examining the development of modulation by the neuropeptide FMRFamide which has inhibitory effects on the pleural SNs in adults. Since FMRFamide and 5HT are known to exert antagonistic effects on the same ionic current through activation of different second messengers, we can use this system to analyze how interacting second messenger pathways are assembled during development.

501.10

IDENTIFICATION OF REINFORCEMENT PATHWAYS NECESSARY FOR OPERANT CONDITIONING IN *APLYSIA*. D.G. Cook & T.J. Carew, Yale University, Department of Psych., New Haven, CT 06520

Aplysia can be operantly conditioned to change their head-waving behavior in order to avoid aversive bright light (Cook & Carew, 1986). A cellular analysis of this conditioning requires the identification of the neural pathways that convey the aversive photic input to the CNS. Previous studies showed that: (1) the primary visual pathways (Optic and Rhinophore nerves [O/R nn.]) mediate *inhibition* of light-induced excitation in pedal (head-waving) motor neurons; and (2) the O/R nn. are *not* necessary for operant conditioning (Cook & Carew, 1987). We now report that the oral veil nerves (C1-C3) mediate light-induced *excitation* of these motor neurons and *are* necessary for operant learning.

We recorded intracellularly from motor neurons using the split-foot preparation (with the O/R nn. cut), and compared the effect of whole-body illumination before and after bilateral cuts of C1-C3. Transection of these nerves abolished the light-induced increase in the motor neuron firing rate (pre cut: $\bar{x}$= +74% increase over baseline firing; post cut: $\bar{x}$= +2%; p<.005; N=6).

Next we assessed the effects of chronic bilateral cuts of C1-C3 on operant conditioning. Three groups were trained: (1) CONTINGENT (O/R nn. cut, N=15); (2) CONTINGENT (O/R *and* C1-C3 nn. cut, N=15); (3) YOKED CONTROLS (O/R nn. cut [N= 7] and O/R & C1-C3 nn. cut [N=8]). Confirming previous results (Cook & Carew, 1987), Contingent Trained animals with only O/R nn. cut showed significant operant learning (p<.025). In contrast Contingent Trained animals which, in addition to O/R nn., also had C1-C3 nn. cut, were comparable to Yoked Controls; neither group showed learning. Thus, nerves C1-C3, which mediate light-induced excitation of pedal motor neurons, are necessary for operant conditioning. It will now be important to identify neurons which have processes in nerves C1-C3, and to characterize their synaptic connections with neural circuits that mediate head-waving.

501.11

DISSOCIATION OF MONOSYNAPTIC AND POLYSYNAPTIC CONTRIBUTIONS TO DISHABITUATION, SENSITIZATION AND INHIBITION IN *APLYSIA*. W.G. Wright*, E.A. Marcus, and T.J. Carew (SPON: S. Tomiko). Depts. Psych. & Bio., Yale Univ., New Haven, CT, 06520

Recent behavioral experiments (Marcus et al., 1988) revealed three different effects of tail shock on the siphon withdrawal reflex of *Aplysia:* (1) rapid facilitation of decremented responses (dishabituation) that is produced by weak but not by strong shock; (2) rapid and transient inhibition of non-decremented responses; and (3) delayed facilitation of non-decremented responses (sensitization) that is only produced by strong shock. We have begun to examine the cellular loci responsible for these behavioral effects in the neural circuit mediating siphon withdrawal.

(1) In a reduced preparation, weak tail-shock produces rapid facilitation of decremented COMPLEX EPSPs (elicited by tactile stimulation of the siphon) in siphon motor neurons (MNs) (med=126% of baseline; p<.02) while strong shock does not (med=112%). In contrast, decremented monosynaptic connections (MONOs) from LE sensory neurons to the same MNs are facilitated by both weak (med=197%; p<.04) and strong (med=188%; p<.02) shock. (2) In the same preparation, strong shock produces inhibition of the non-decremented COMPLEX with a time course paralleling behavioral inhibition; this inhibition is not observed at the level of the MONO (Wright et al.,1988). (3) In a whole-animal preparation, strong shock has no immediate effect on the amplitude of the COMPLEX but produces significant facilitation 10 min after shock (med=130%; p<.03), which grows to a maximum at 30 min (149%; p<.01). This delayed facilitation is again not observed in the MONO.

We have shown that tail shock differentially affects the COMPLEX and MONO, and that changes in the COMPLEX more closely parallel the behavioral effects of tail shock. These results suggest that interneurons and/or novel sensory neurons may account for the diverse effects of tail shock in this reflex.

501.12

SEROTONIN DIFFERENTIALLY MODULATES MONOSYNAPTIC AND COMPLEX EPSPS IN SIPHON MOTOR NEURONS IN APLYSIA. K. Fitzgerald* and T.J. Carew (SPON: R.J. Wyman). Department of Psychology, Yale University, New Haven, CT 06520.

Tail shock produces initial transient inhibition prior to the delayed onset of sensitization in the siphon withdrawal reflex of *Aplysia* (Marcus, et al., 1988). This inhibitory effect is reflected in the complex EPSP in siphon motor neurons (MNs), with a time course paralleling the behavior; but the monosynaptic EPSP from siphon sensory neurons (SNs) does not parallel the behavior (Wright et al., 1988). We here report that, like tail shock, serotonin (5HT) has differential effects on monosynaptic and complex EPSPs in siphon MNs.

Intracellular recordings were made from SNs and MNs in the isolated abdominal ganglion. Both monosynaptic (MONO) EPSPs (elicited by injecting current into SNs) and COMPLEX EPSPs (elicited by shock to the siphon nerve) were tested at 10 min intervals. Two pre-tests were given to determine baseline EPSP amplitude. 5HT (50 μM) was then bath-applied for 2 min, beginning 7.5 min after the last pre-test. In a test 30 sec after the end of application, 5HT produced significant *facilitation* of the MONO EPSP (mean EPSP amplitude= 190% of baseline, p<.01, n=11) and simultaneous *depression* of the COMPLEX EPSP (mean=76%, p<.01). Thus, the transient inhibitory effect of 5HT mimics the effect of tail shock on the COMPLEX EPSP.

To determine the specificity of the 5HT effects, we have begun to examine other endogenous neurotransmitters. For example, the peptide SCP_B (10 μM; n=10) does not produce inhibition of the COMPLEX EPSP, but rather a modest (non-significant) increase in both the MONO and COMPLEX EPSPs. The effect of SCP_B on the COMPLEX was significantly different from that of 5HT (p<.005).

These results suggest that modulatory neurotransmitters can affect elements in the siphon withdrawal circuit differentially. It will now be important to identify the different sites of action of these transmitters, and determine the degree to which their effects contribute to the multiple components of plasticity observed at both behavioral and synaptic levels.

PAIN PATHWAYS

502.1

AFFERENT C-FIBERS FROM THE RAT HAIRY SKIN NOT DRIVEN BY NATURAL STIMULATION. H.O. Handwerker*, S. Kilo* and P.W. Reeh*. (SPON: D. Bonke). Dept. Physiol. Biokybern., Erlangen University, D-8520 Erlangen, FRG

Single afferent units were recorded from dorsal rootlets which were driven by electrical stimulation of the sural nerve in anaesthetized rats. The other hindleg nerves were cut. Weak and noxious mechanical stimuli, radiant heat pulses and cold stimuli were used for searching receptive fields in the skin. 49/52 A-beta and 15/18 A-delta units were driven by mechanical stimulation from their receptive fields, half of the A-delta units being high threshold mechanoreceptors. In contrast, only 26/50 C-fibers could be driven by skin stimulation, most of them being nociceptors. Conduction velocities and electrical thresholds of responsive and non-responsive C-fibers were not significantly different. When we only considered C-fibers recorded when the skin had not been manipulated too much (first two filaments), signficantly fewer C-fibers (6/21) were responsive (p<0.005). We conclude, that at least some of the unresponsive C-fibers are "silent" nociceptors recruited only when their receptive endings are sensitized by inflammatory processes.

502.2

VISCERO-SOMATIC CONVERGENCE IN THE MEDIAL REGION OF THE CAT'S THALAMUS. J. Brüggemann*, A.V. Apkarian, M.K.C. Mengel*, D.F. Cechetto, C. Vahle-Hinz and K.-D. Kniffki. Physiologisches Institut der Universität, D-8700 Würzburg, F.R.G.

Single and multi unit recordings were performed in pentobarbital-anesthetized cats. Thalamic responses to electrical (vagus and splanchnic nerves; V, S) and natural visceral (taste, chemo- and baroreceptor activation; esophagus, bladder and/or colon distension) and somatic (touch, pinch, heat) stimuli were mapped in nuclei surrounding the ventral posteromedial n. (VPM).

For both nerves, the distribution of the response loci was similar for ipsi- and contralateral thalami. Of the sites with electrically elicited responses 22% (19/86) were found in the periphery of VPM (VPM_p) and none in VPM proper; 33% were located in medial dorsal n. (MD), centre médian (CM) and central lateral n. (CL); 16% were found in zona incerta (ZI). 29% of the recording sites were sparsely distributed over adjacent nuclei. MD, VPM_p and ZI had mostly S inputs, while other regions showed responses from both nerves. Thirty-one units responded to natural visceral stimuli; 16 out of 20 tested had somatic nociceptive responses as well, whereas only 2/21 had input from low-threshold mechanoreceptors. Five of the units driven by natural visceral stimulation were located in VPM_p and none in VPM proper, 14 were found in MD, CL and CM, and 3 in ZI. The dominant visceral input for MD units derived from baro- (9/11) and chemoreceptors (7/9), and 5/11 MD units had input from two or more types of visceral receptors.

The results indicate that the medial region of the thalamus is involved in visceral sensation and that there is strong convergence of visceral and, almost exclusively nociceptive, somatic inputs.

502.3

RETROGRADE LABELING OF SPINAL CORD NEURONS THAT PROJECT TO NUCLEUS SUBMEDIUS IN THE RAT. R.J. Dado and G.J. Giesler, Jr. Dept. of Cell Biol. and Neuroanat., Grad. Prog. in Neuroscience, Univ. of Minn., Minneapolis, MN 55455.

Previous studies using anterograde labeling in rats, cats and monkeys have shown spinal and trigeminal projections to the thalamic nucleus submedius (Sm; Craig and Burton, 1981). In the cat, the input to Sm was shown to originate predominantly in the marginal zone in the spinal cord and medulla. The purpose of the present study was to label the neurons that project to Sm in the rat. Small iontophoretic injections of Fluoro-Gold (FG) were centered in Sm in five rats. After 3-5 days, 18 identified spinal segments and caudal brainstem were sectioned. In alternate sections, an average of fewer than 100 labeled spinal cord neurons were counted in each rat. A total of only 2 neurons were labeled in the marginal zone in these five rats. The majority of labeled spinal neurons were in the deep dorsal horn. Sixty-one percent of the labeled neurons in the spinal cord were contralateral. In contrast to labeling in the cord, a total of 179 marginal zone neurons were labeled in nucleus caudalis in the five cases. Control injections of the same size (n=3) in the ventrobasal complex labeled an average of more than 400 spinal cord neurons. A control injection into the posterior thalamic nucleus labeled more than 700 neurons in the spinal cord, including 110 in the marginal zone. Thus, the results of our control injections provide evidence that iontophoretic injections of FG can label many spinal neurons including those in the marginal zone. These results indicate that the spinal cord projection to Sm in the rat is small and does not originate from marginal zone neurons. Supported by NS25932 and DA07234.

502.4

Ultrastructure of Tooth Pulp and HTM-tongue Terminations in Trigeminal Interstitial Nucleus of the Cat. R.C. Shults, A.M. Kavookjian*, and A.R. Light (Depts of Physiology and Orthodontics, UNC-Chapel Hill, Chapel Hill, NC 26599-75435)

Physiologically identified single nociceptive-specific primary afferent fibers innervating tooth pulp or tongue were stained intra-axonally with HRP at the level of obex. Both types of fibers distributed *en passant* and terminal synaptic boutons to the interstitial nucleus in the spinal trigeminal tract and to selective portions of the trigeminal spinal nuclei. *Tooth pulp* terminal profiles in the interstitial nucleus contained numerous mitochondria, clear, round vesicles, and large, dense-core vesicles. Large, dense-core vesicles were not visualized in every section, but were numerous when present. Tooth pulp afferents formed asymmetric presynaptic contacts on small dendritic profiles and spine heads, and were postsynaptic to axonal profiles. An occasional contact was identified on the soma of cells in the interstitial nucleus.

HTM-tongue terminal profiles contained numerous mitochondria and clear, round vesicles. In contrast to tooth pulp, only a few large, dense-core vesicles were identified. HTM-tongue terminals made asymmetric presynaptic contacts on small dendritic profiles and were postsynaptic to axonal profiles. Ultrastructural characteristics of HTM-tongue terminal profiles, but not tooth pulp, are similar to cutaneous HTM (Rethelyi et al., 1982). Supported by PHS grants #DE00169 and #NS16433.

502.5

FIRING FREQUENCY OF HUMAN C POLYMODAL NOCICEPTORS: THE EFFECT OF RATE OF RISE OF NOXIOUS HEAT. D.Yarnitsky*, D.Simone, R.Dotson, M.A.Cline, and J.L.Ochoa. Dept.of Neurology, Good Samaritan Hosp.& Med.Ctr., Oregon Health Sciences University,Portland,OR,97210 USA.

Dependence of firing frequency on rate of temperature (T) change is known for warm and cold-specific primary afferents; the higher the rate, the higher the frequency. It is unknown whether firing of C polymodal nociceptors is also influenced by rate of T change. To study this, heat stimuli were given through a Peltier thermode to hairy skin of hand or foot in normal volunteers. Starting at adapting T of 32°C, ramps of fixed amplitude (ad hoc per subject) were given at 3 different rates of T rise, (common to all subjects). Action potentials from single C polymodal nociceptor afferents ($\bar{CV}$ 0.72 m/sec,N=10) were recorded using microneurography. Firing frequency increased significantly as a function of increase in rate of T change. Average frequencies were 1.17, 4.98, and 12.94 Hz for rates of 0.32, 2.3 and 6.7° C/sec, respectively ($p<0.001$, Repeated Measures ANOVA).

The present data supports the previous conclusion that, as measured through the method of limits, increase in heat pain thresholds with rate of T change is artifactual (Yarnitsky & Ochoa, Abstr Soc Neurosci, 1988). At higher rates of T rise, nociceptor firing sufficient to induce pain sensation is reached earlier along the T ramp, such that perceptive threshold decreases, as truly revealed by the forced choice method.

502.6

PRIMATE MODEL OF TONIC PAIN, PAIN TOLERANCE AND COUNTERIRRITATION M.C. Bushnell, S. Marchand, G.H. Duncan and N. Trudeau*. Cen. Rech. Sci. Neurol., Univ. Montréal, Canada H3C 3J7.

Animal pain models that parallel human models allow the study of pain and analgesia under conditions in which the human perception is known and provide a clinical relevance that is frequently questioned with such tests as rodent tail flick and hot plate reaction. Also, by using painful stimuli tolerated by humans and allowing the control of stimuli that is customary for human subjects, the ethical treatment of the animals is better insured. A pain model frequently used in humans is the cold pressor test, which approximates clinical acute pain and produces a tonic pain for studying counterirritation. In the present study we have developed a primate cold pressor model that parallels that used in humans.

One adult female macaque received a liquid reward for pressing a button positioned at the bottom of a pan of refrigerated water. The monkey received a reward every 5 s while depressing the button, but had to wait 90 s before initiating a new trail after releasing the button. The time the monkey kept its hand in the water (withdrawal latency) was compared for water temperatures of 0°C, 10°C, and 35°C.

The monkey's withdrawal latency varied directly with water temperature (34 s at 0°C, 78 s at 10°C and 180 s at 35°C, $p<0.02$). Further, withdrawal latency was influenced by motivational level, as is usually true for pain tolerance measures. At each water temperature, withdrawal latencies were longer during trials early in a session, when the monkey had not received fluids for 12 h, than at the end, when the monkey was satiated. Pain thresholds from two humans in a similar task were shorter than the monkey's withdrawal latency (17.5 s at 0°C and 51.2 s at 10°C), but tolerance time was longer than that of the monkey (51 s at 0°C and 218 s at 10°C).

The present data suggest that the cold pressor test can be used successfully in monkeys as a pain model for the assessment of analgesic treatments and the study of endogenous pain-modulatory pathways.

502.7

HIPPOCAMPAL SYMPATHETIC INGROWTH AND PHOSPHOINOSITOL METABOLISM. L.E. Harrell, D.J. Connor and D.S. Parsons.* Dept. of Neurology, VA and University of Alabama Med. Ctr., Birmingham, Ala. 35294

Following cholinergic denervation of the hippocampus, via medial septal lesions (MSL), peripheral sympathetic fibers, originating from the superior cervical ganglia, grow into the hippocampus. Since both muscarinic and adrenergic receptors have been linked to phosphoinositol (PI) hydrolysis, we attempt to define the functional integrity of the hippocampus after MSL and sympathetic ingrowth (SI) by the measurement of carbachol and norepinephrine (NE)-stimulated PI hydrolysis. Thirty adult male rats underwent one of three surgical procedures: Con (sham surgery); MSL (ingrowth animals); MSL+Gx (ganglionectomized). Three months later the animals were sacrificed, the hippocampi removed, and PI hydrolysis assessed in the presence of carbachol (50uM-5mM) and NE (1, 5, 200uM). Carbachol was found to enhance PI hydrolysis over basal levels in all three groups in a dose-dependent fashion. With maximal stimulation (5mM), PI hydrolysis was found to be significantly ($p < .04$) greater in the MS group (13.3 ± 1.1 $IP_1/(IP_1 + Lipid)$ when compared to both the CON (9.8 ± 1.2) and MS+Gx (11.4 ± 1.2) groups. Similarly, NE was also found to enhance PI hydrolysis in a dose dependent manner; however, no differences were observed among the three groups. These results suggest that SI is capable of altering hippocampal functional integrity through cholinergic mechanisms.

502.8

MORPHINE APPLIED TO HUMAN PERIPHERAL NERVES DOES NOT CAUSE ANALGESIA TO CUTANEOUS PAIN STIMULATION.

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Background. Opioid receptors have been demonstrated on human primary afferents but there are conflicting findings concerning their role as pain modulators.

Method. In experiment 1 ten ml morphine hydrochloride (4%) or ten ml isotonic saline were injected perineurally to the ulnar nerve. The pain threshold to argon laser stimulation was measured before injection and 5, 10, 15, 30, 45, 60, 90 and 120 min. after injection. In experiment 2 the arm was exsanguinated. After inflation of the cuff around the upper arm, 40 ml morphine hydrochloride (0.02%) or 40 ml isotonic saline were injected intraveneously. The cuff was deflated after 30 min. The pain threshold was measured before injection and 5, 10, 20, 25, and 30 min. after injection, and 5, 10, 15, and 30 min. after deflation of the cuff. Ten healthy volunteers participated in each experiment. Injections were performed double blind.

Results. In either of the experiments no significant differences in pain thresholds were observed between morphine and saline injections. After 30 min. of ischemia, total analgesia to laser induced pricking pain was obtained.

Conclusion. For the morphine concentrations considered in the present study, the opioid receptor does not contribute to modulation of pricking pain.

503.1

GLIAL FIBRILLARY ACIDIC PROTEIN (GFAP): SEQUENCE AND REGULATION OF THE HUMAN GENE. M. Brenner*, Y. Nakatani*, C. Banner*, E.T. Browning and E. Freese. Lab. of Molec. Biol., NINDS-NIH, Bethesda, MD 20892.

A complete genomic clone has been obtained for human GFAP, the major component of intermediate diameter filaments of mature astrocytes. The nucleic acid sequence obtained for the coding region and intervening introns shows considerable similarity to that previously determined for the mouse gfa gene. The mRNA start site, determined by primer extension and probe protection experiments, is also similar to that found for the mouse. By contrast, the initiating ATG for human gfa, determined by a novel in vitro transcription and translation method, leads to a deduced amino terminal sequence for human GFAP that differs markedly from that predicted for the mouse. This discrepancy was resolved by discovering that the published mouse nucleic acid sequence has an incorrect additional base.

The region around the mRNA start site contains sites similar to consensus sequences for binding of several transcription factors, including those for AP-2, the TATA factor, and the Box A factor. The simultaneous presence of the latter two sites is unusual, as the TATA sequence is commonly part of promoters utilizing RNA polymerase II (Pol II), while the Box A sequence is commonly used by Pol III. Our studies show that both elements can independently direct proper initiation of human gfa transcription by Pol II, and that the sites likely interact synergistically.

503.2

LOCALIZATION OF MEC-3 EXPRESSION USING A MEC-3-LACZ GENE FUSION IN CAENORHABDITIS ELEGANS. Jeffrey C. Way[1,2] and Martin Chalfie[2], 1) Biology Dept., Princeton Univ. Princeton, NJ 08544, and 2) Dept. Biological Sciences, Columbia University, New York, NY 10027

mec-3 is a homeobox-containing gene required for expression of differentiated characteristics of the touch receptor neurons of the nematode Caenorhabditis elegans (Way and Chalfie, Cell 54, 5). To identify the cells in which mec-3 is expressed, we constructed a translational fusion between the mec-3 homeobox and lacZ, and injected it into C. elegans.

When transformed animals are stained with X-gal the touch receptors are stained, as predicted. In addition, the FLP and PVD neurons also stain. We identified these cells by the pattern of DAPI-stained nuclei in X-gal-stained animals, and by the absence of stained cells in the unc-86 mutant, which lacks these cells due to lineage alterations.

The PVD neurons appear to be mechanoreceptors that sense a harsher touch stimulus. Elimination of these cells by either mutation or laser ablation abolishes the response to prodding in the center of the body (assayed in animals that lack the touch receptors).

The pattern of expression of the mec-3-lacZ fusion in mutant strains indicates that the homeobox-containing gene unc-86 is necessary for all mec-3 expression, and that mec-3 turns on its own synthesis.

503.3

HERPES SIMPLEX VIRUS TYPE 1 LATENCY-ASSOCIATED TRANSCRIPT EXPRESSION IN VITRO. W. Williamson* and B. Wigdahl (SPON: R. Ziegler). Department of Microbiology and Immunology, The Pennsylvania State University College of Medicine, Hershey, PA 17033.

Herpes simplex virus type 1 (HSV-1) is maintained in a latent form in neurons of the peripheral sensory ganglia. Fraser and coworkers (J. Virol. 55:849-852, 1985) have demonstrated that during latent infection of neurons the HSV-1 genome most likely exists as a unit-length circular molecule. Studies by a number of investigators have shown that few, if any, virus genes are expressed during latency. However, hybridization analyses of RNA isolated from mouse trigeminal ganglia have resulted in the detection of a viral RNA species that accumulates throughout the course of latent infection. This transcript, termed the latency-associated transcript (LAT), accumulates to high levels primarily in nuclei of latently infected neurons (Stevens et al., Science 235:1056-1058, 1987). Although dispensable for the establishment of latency, HSV-1 LAT may be required for the stable, long-term maintenance of latency (Javier et al., Virology 166:254-257, 1988). To examine the differential expression of HSV-1 LAT in vitro we subcloned the DNA sequences upstream from the HSV-1 transcriptional start site. Hind III linkers were added to these cloned fragments and the latter were transferred into a pSV0-CAT expression vector system for use in transient expresssion assays in a series of human and mouse neuroblastoma, glioblastoma, and fibroblastoid cells. Because HSV-1 LAT is preferentially expressed at high levels only in latently infected neurons, it is reasonable to hypothesize that chloramphenicol acetyl-transferase expression driven by regulatory elements of HSV-1 would be significantly higher in neuronal cells. Studies are currently directed at the identification of transcriptional factors that may be involved in the up-regulation of LAT expression in neurons.

503.4

SCIP-1: A cAMP-INDUCIBLE MEMBER OF THE POU TRANCRIPTION FACTOR FAMILY EXPRESSED IN SCHWANN CELLS. E.S.Monuki*, G.A.Weinmaster*, and G.E.Lemke, Molecular Neurobiology Laboratory, The Salk Institute, La Jolla, CA 92037.

The factors involved in the regulation of myelin gene expression in the mammalian nervous system are currently unknown. In an attempt to isolate and characterize these factors, degenerate synthetic oligonuclcotides were constructed to conserved regions of the POU family of cell-specific *trans*-acting proteins. These oligonucleotides were used to screen a rat Schwann cell cDNA library, and a positive clone has been isolated and purified. Sequence analysis has revealed that this cDNA represents a novel POU factor that contains the conserved homeodomain and POU-specific regions characteristic of this family. Northern analysis suggests that this factor is expressed in a Schwann cell-specific manner. We have named the factor SCIP-1 (pronounced "skip").

Since *in vitro* studies suggest that cAMP is the key second messenger involved in regulating Schwann cell myelin gene expression *in vivo*, we investigated the role of cAMP in SCIP-1 expression. Northern analysis indicates that SCIP-1 mRNA is expressed at high levels in actively-myelinating Schwann cells present in the neonatal rat and is strongly induced by the adenyl cyclase activator forskolin in cultured Schwann cells in a dose-dependent fashion. Kinetic analysis of this cAMP-mediated induction reveals that SCIP-1 is expressed only after the repression of the immediate early proto-oncogene, c-jun, and precedes the expression of the myelin structural genes such as P0 and MBP. SCIP-1 (which stands for Schwann Cell Inducible POU) may therefore represent an intermediate factor in the regulatory cascade of *trans*-acting factors that ultimately results in the formation of myelin *in vivo*.

503.5

MOLECULAR BIOLOGICAL INVESTIGATION OF THE SUBSTANTIA INNOMINATA. CHARACTERIZATION OF A 15.5KB BRAIN SPECIFIC GENE. B.E.Boyes, D.G.Walker* P.L.McGeer and E.G.McGeer. Kinsmen Lab. of Neurological Research, University of B.C., Vancouver, B.C. Canada.

An apparent selective loss of the magnocellular cholinergic neurons of the basal forebrain is observed as part of the pathology associated with Alzheimer disease (AD). A majority of the neocortically projecting magnocellular cholinergic neurons are located in the substantia innominata (SI).

A plasmid cDNA library of genes from a normal SI was constructed and screened by a negative selection procedure to remove genes that are common to the cerebellum. The remaining clones were screened by differential hybridization with cDNA probes of normal and AD affected SI. From these, a 3 kbp clone was identified that recognizes a large transcript (15.5 kb). RNA hybridization analysis revealed that this gene is expressed in a number of brain regions (temporal and occipital cortex, hippocampus, caudate putamen, cerebellum and SI), but not in white matter, liver or placenta. Low stringency hybridization to RNA from rat also revealed a similar large transcript in RNA samples from different brain regions and adrenal gland, but not in muscle or peripheral organs. Homology between the human and rat genes appeared to be low. The DNA sequence obtained from this clone did not have detectable homology with any sequences in the Genbank DNA sequence database.

503.6

INTERACTION OF SPIN-LABELED CALMODULIN VARIANTS WITH AXONAL PROTEINS. D.T. Rivera*, G.F. You* and D.J. Nelson* (SPON: T. Schoenfeld). Dept. of Chemistry, Clark Univ., Worcester, MA 01610.

Calmodulin (CaM), the principal eukaryotic intracellular receptor for calcium ion, is known to be involved in the regulation of many neurobiological functions controlled by calcium ion. For example, calci-calmodulin has been shown to stimulate the phosphorylation of a number of proteins in synaptic vesicles, presumably by binding to and activating a synaptic Ca(II)-kinase. CaM has also been implicated in neurotransmitter release processes, presumably by promoting synaptic vesicle-membrane interactions. Operating under the working hypothesis that CaM is the key agent which mediates many intra-axonal processes critical to neurotransmitter release, we have been investigating the mechanism by which CaM physically interacts with its "targets: (both metal ions and enzymes) in the nerve cell, utilizing electron paramagnetic resonance (EPR) spectroscopy (see J. Inorg. Biochem. 33, 139-147, 1988). Part of this effort involves the production of bovine calmodulin variants, by employing the techniques of site-directed mutagenesis, which will facilitate the attachment of specific EPR probes to critical regions of the CaM molecule, such as at the proposed target enzyme binding site in the central helical region.

503.7

EXPRESSION AND CHARACTERISATION OF THE 50 kDa SUBUNIT OF Ca^{2+}/CALMODULIN-DEPENDENT PROTEIN KINASE II (CK-II) IN EUCARYOTIC CELLS. J.ARONOWSKI*, P.KELLY AND N.WAXHAM. (SPON: G. CRAVISO). Depts.of Neurology and Neurobiology and Anatomy, Univ. of Texas Medical School, Houston, TX 77030.

A rat brain cDNA encoding the 50 kDa (alpha) subunit of CK-II has been expressed in eucaryotic cells. The cDNA was inserted into a heat-shock inducible vector and used to stably transfect CHO cells. Ca^{2+}/calmodulin (CaM)-dependant phosphorylation of transfected and heat-shocked CHO cell homogenates showed that two major proteins were phosphorylated. The first phosphoprotein was a 50 kDa polypeptide recognized by monoclonal antibodies specific to the 50 kDa subunit of rat forebrain CK-II. Additionally, two dimensional tryptic maps of ^{125}I-labeled peptides of CHO-expressed 50 kDa protein and the 50 kDa subunit of rat purified CK-II showed the same pattern, confirming that the expressed-50 KDa protein represented the alpha subunit of rat brain CK-II. No immunodetectable or autophosphorylation detectable 50 kDa protein was present in nontransfected CHO cells. The second phosphoprotein was approximately 58 kDa and migrated on two-dimensional isoelectric focusing /SDS-PAGE in a fashion similar to tubulin. Purification of the 50 kDa protein from CHO cells by CaM-Sepharose chromatography yielded a 60-70% pure kinase. Time dependent phosphorylation as well as the amount of CaM required to half maximally activate both enzymes were indistinguishable. Km values for syntide phosphorylation were approximately 2-3 uM and Vmax values were 1.3-1.5 umol/min/mg for both enzyme preparations. Like rat forebrain CK-II, after Ca^{2+}/CaM-dependent autophosphorylation, the expressed-50 kDa subunit no longer required Ca2+/CaM for further auto- and substrate-phosphorylation. The role of phosphorylation on translocation of the 50 kDa subunit was also studied.

503.8

CLONING OF THE CDNA ENCODING THE LIMBIC SYSTEM-ASSOCIATED MEMBRANE PROTEIN (LAMP). K. Pennypacker, B. Bhardwaj*, P. Levitt and B. Schepart*. Depts. of Anatomy and Microbiology, Medical College of Pa., Phila., Pa. 19129

LAMP is a novel membrane-associated glycoprotein that appears to be involved in the target recognition of the limbic system and its connections. In order to further define the function and structure of LAMP, we have utilized recombinant DNA technology. Using a LAMP-specific monoclonal antibody, we have isolated several clones from a rat brain cDNA expression library at a frequency of 10^{-4}. One representative clone, KP6, with a 3.2 kb insert, has been partially nucleotide sequenced. The 5' end of approximately 300 nucleotides encode an open reading frame (ORF), with the first 30 amino acids being hydrophobic and possibly encoding a signal sequence. Additional regions appear as ORFs over the approximate next 2000 nucleotides, encoding predominantly hydrophilic amino acids. Thus far, the sequence is unique to other published sequences except that it contains an ALU repetitive sequence which is homologous to many other cDNAs. Initial northern blot analysis revealed several distinct bands with polyadenylated RNA from the brain but not liver suggesting that the message is from a multigene family or differentially spliced. Supported by March of Dimes Basic Research Grant 1-9119.

503.9

FUNDAMENTAL MECHANISMS OF TUMORIGENESIS IN ASTROCYTOMAS. R.Chung*,M.El-Azouzi*,C.Hettlich*,G.Farmer*,K.Anderson*, P.McL.Black*,T.Hedley-Whyte,J.Gusella,R.Martuza, and B. Seizinger*. Massachusetts General Hospital and Harvard Medical School, Boston, MA 02114.

Astrocytomas, including glioblastoma multiforme, represent the most frequent and deadly neoplasias of the central nervous system. Despite numerous cytogenetic and oncogene studies, the primary mechanism of tumorigenesis in astrocytic tumors remains obscure. Recent evidence suggests that the loss or inactivation of certain "tumor suppressor" loci leads to the formation or a variety of human cancers, e.g. retinoblastoma and acoustic neuroma.

For this reason, fourteen astrocytomas of varying grades of malignancy were analyzed with a battery of polymorphic DNA markers to search for specific chromosomal deletions. Although not the only genetic aberration seen, the most commonly detected losses were found for loci on the short arm of chromosome 17. This region does not include the gene causing Von Recklinghausen's neurofibromatosis (NF 1).

Interestingly, loss or inactivation of both copies of p53, a putative "tumor suppressor" gene located on chromosome 17, has recently been implicated in the tumorigenesis or colon carcinoma. The possible involvement of p53 as a candidate locus in astrocytoma development is currently studied. Characterization of this and other "tumor suppressor" loci may shed insight into fundamental mechanisms of tumorigenesis in the central nervous system.

503.10

EVOLUTION OF SYNAPTOSOMAL-ASSOCIATED PROTEIN-25 (SNAP-25): SEQUENCE IDENTITY OF MOUSE AND CHICKEN PROTEINS AND SIMILARITY TO DROSOPHILA PROTEIN. D. Larhammar, A. Blomqvist*, S. Catsicas§, R. J. Milner§, and M. C. Wilson§. Dept of Medical Genetics, Uppsala University, S-751 23 Uppsala, Sweden, and §) Research Institute of Scripps Clinic, La Jolla, CA 92037.

SNAP-25 is a 25-kD protein which is expressed exclusively by some neuronal subpopulations. It is localized to presynaptic nerve terminals and is associated with synaptosomal membranes. Its onset of expression correlates precisely with the time of synaptogenesis.

SNAP-25 cDNA clones were originally isolated from mouse. We have used a mouse probe to screen a chick retina cDNA library made from mRNA of embryonic day 15. Numerous clones were identified and indicated an mRNA abundance of 0.1% at this developmental stage. The predicted protein sequence is 100% identical to murine SNAP-25 throughout the 206 amino acids. The only other non-DNA-binding proteins that show perfect identity between these species are α-actin, calmodulin and ubiquitin. Also the 3'-untranslated region of SNAP-25 mRNA shows significant similarity between mouse and chicken.

Southern hybridizations to genomic DNA of several animal species showed conservation of SNAP-25 throughout the vertebrates. Also Drosophila melanogaster displayed cross-hybridizing bands. Therefore, a D. melanogaster adult head cDNA library was screened with a mouse SNAP-25 probe. Several clones were isolated and indicate an mRNA abundance of 0.01%. These clones are in the process of being characterized.

The absolute sequence identity of SNAP-25 between chicken and mouse indicates that this protein subserves evolutionarily old and important functions, and that it presumably has multiple points of interaction with other proteins.

503.11

L7 GENE STRUCTURE AND THE ISOLATION OF A PUTATIVE PURKINJE CELL PROMOTER/ENHANCER. J. Oberdick*, L. Sangameswaran*, J. Hempstead*, A. Berrebi‡, E. Mugnaini‡ and J.I. Morgan* (SPON: R. Chizzonite). Roche Institute of Molecular Biology, Roche Research Center, Nutley , N.J. 07110 and ‡University of Connecticut, Storrs, CT, 06269.

L7 is a gene previously reported to be exclusively expressed in cerebellar Purkinje cells (Oberdick, J. et al, *Neuron*, 1:367, 1988), but which we now show also to be expressed in a restricted population of retinal neurons, the bipolar cells. Several other markers isolated in our lab, namely PEP-19 (Ziai, R. et al, *PNAS*, 83:8420, 1986) and cerebellin (Slemmon, J. et al, *PNAS*, 82:7145, 1985), share the Purkinje cell restriction within the cerebellum, but all three markers have unique localization patterns throughout the remainder of the brain. L7 and cerebellin are the most restricted, while PEP-19 is more widely distributed in many brain regions. One of the goals of our research is to delineate regions of control within the respective marker genes which 1) provide Purkinje cell specificity and 2) control their unique spatial and temporal patterns. One might expect the former to be reflected in shared elements within marker gene promoter sequences, the latter in differences. As a first step in this analysis we report the cloning and sequencing of L7 genomic DNA from rat and mouse, and the identification of non-coding regions which are highly conserved between the two species. Experiments utilizing various L7 promoter constructs have been undertaken and will be discussed. In addition we have found that L7 protein undergoes post-translational processing which is differentially regulated during Purkinje cell development resulting in two forms of L7 in adults. Only one form of L7 appears in the retina. At least one mutation affecting Purkinje cell development in mouse disrupts this processing. Experiments designed to identify this tissue specific and temporally regulated modification process have been initiated.
Partially supported by PHS Grant #09904.

503.12

NEURONAL GROWTH RESPONSE GENES EXPRESSED IN CANARY SONG CONTROL REGION HVC: CLONING OF EGR-1/NGFI-A HOMOLOGUES. C. Mello*, M. Heucas* and D.F. Clayton, Lab. of Animal Behavior, The Rockefeller Univ., NY NY 10021

We are trying to identify and analyze genes which act as primary regulators of neuronal plasticity, and have been focusing on the higher vocal center (HVc) of canaries. HVc undergoes significant seasonal and steroid-regulated changes in volume in adulthood, reflecting changes both in neuritic outgrowth and neuron number. Egr-1/NGFI-A is a candidate primary regulator gene, isolated in mammals by several investigators independently (Cell 53:37, Science 238:797, PNAS 85:4691). It is induced rapidly following stimuli for neuronal growth and development, and encodes a protein with the "multiple zinc finger" structure believed to be characteristic of a class of transcriptional regulators. To look for expression of homologous genes in HVc, we screened an HVc cDNA library at low stringency with cDNA clones provided by V. Sukhatme (mouse Egr-1) and J. Milbrandt (rat NGFI-A). We have identified at least one clone with a high degree of sequence similarity in the HVc library, and are conducting further analyses. We speculate that the expression of an Egr-1/NGFI-A homologue in HVc may serve an important functional role in regulating other genes involved in the plasticity of this brain region.

503.13

GENOMIC CLONING OF MOUSE NA^+, K^+-ATPASE $\alpha 1$, $\alpha 2$, AND $\alpha 3$ GENES. See-Ying Tam, Edwin N. Geissler*, Sharon L. Graw* and David E. Housman*. Center for Cancer Research, M.I.T., Cambridge, MA 02139.

The α subunit of the Na^+, K^+-ATPase contains the catalytic site for ATP hydrolysis and the binding site for cardiac glycosides. Three isoforms of the α subunit are encoded by three distinct genes. To analyze the transcriptional control of these genes, we have isolated cosmid clones of the genomic sequences of the mouse $\alpha 1$, $\alpha 2$ (αIII), and $\alpha 3$ (α+) genes, average 40 kb in length, in the vector pWE15. Restriction mapping and Southern blot analysis using cDNA probes showed that two overlapping cosmids which span the entire coding region of the $\alpha 1$ gene contain a common 5 kb $\alpha 1$ genomic sequence. The mouse $\alpha 1$ gene is estimated to be 30 kb in length. Co-transfection of the two cosmids into ouabain-sensitive CV-1 monkey cells by calcium phosphate procedure confers ouabain resistance to these cells, with an average yield of 72 resistant colonies/10^6 cells/ug DNA. No ouabain-resistant colonies were observed with transfection of either cosmid alone. Subsequent Southern analysis of the transformants demonstrated that the two cosmids have integrated into the CV-1 cell chromosomes and undergone homologous recombination in the overlapping region to form a functional mouse $\alpha 1$ gene. Functional and DNA sequence analyses of the transcriptional control elements for these three genes are currently in progress.

503.14

IDENTIFICATION OF RETINA-SPECIFIC BINDING SITES IN THE RAT OPSIN PROMOTER M.A. Morabito* and C.J. Barnstable. Yale University School of Medicine, Dept. of Ophthalmology and Visual Science, New Haven CT 06510.

The opsin gene is expressed postnatally in the rod photoreceptor cells and is developmentally regulated at the transcriptional level (Treisman J.E. et al., Mol. Cell. Biol. 8:1570-79,1988). Opsin transcripts can first be detected at PN1 and the rate of transcription increases 30 fold to reach adult levels at PN10.

A 6.5 Kb EcoRI fragment isolated from a rat genomic DNA library contains the complete coding sequence and the promoter region of the rat rod opsin gene. Like other mammalian opsin genes the structural portion consists of five exons interrupted at identical sites by four introns. The coding portion of the gene is highly homologous to the corresponding regions of other mammalian rod opsin genes (86-90%) and these values are even higher when the aminoacid sequences are compared. The homology in the promoter is limited to small regions of DNA like the TATA and CAAT boxes.

The 500 bp of the promoter region proximal to the transcriptional start site of the rat opsin gene contains four CAAT box regions identified by homology with the consensus sequence CAAT and two of these are flanked by short direct repeats. We have previously shown that the CAAT box located at position -121 interacts with a retina nuclear extract to form a unique DNA-protein complex not present when using extracts from other CNS regions. We have now identified two more regions of the rat opsin promoter that bind specifically to the retina extract forming unique DNA-protein complexes not present using brain, cerebellum and liver extracts.

Supported by NIH grants EY05206 and NS20483.

503.15

STRUCTURAL AND POTENTIAL REGULATORY DOMAINS OF A RAT GAP-43 GENE. Elly Nedivi*, Gurigbal S. Basi and J. H. Pate Skene. Dept. of Neurobiology, Stanford University, Stanford, CA 94305.

GAP-43, an abundant growth cone membrane protein, is encoded by a single-copy gene whose transcription is strongly correlated with axon development and regeneration (Cell 49: 785,1987). We have isolated coding and non-coding portions of the rat GAP-43 gene to investigate the organization of protein-coding structural domains, and DNA sequences that may contribute to the regulation of GAP-43 expression. Restriction mapping of rat genomic DNA, and sequence analysis of cloned genomic DNA, show that the GAP-43 gene contains three coding exons. The 5' exon (Exon 1) encodes a small (10 aa) amino-terminal domain containing the proposed site for fatty acylation and membrane binding of the protein. Exon 2 codes for the majority of GAP-43, including the proposed calmodulin-binding site and the principal site phosphorylated by protein kinase C. The 3' exon codes for a small (27 aa) carboxy-terminal domain with partial homology to neurofilament proteins. One interpretation of this organization is that GAP-43 comprises a core domain that interacts with intracellular messenger systems, bounded on either end by domains that position the protein between growth cone membranes and the submembranous cytoskeleton. Sequence analysis of the GAP-43 gene 5' to the coding regions reveals a 68 base pair sequence capable of forming H-DNA (a triple- and single-stranded right-handed conformation) and a second sequence of 34 bases with the potential to form the left-handed Z-DNA conformation. Unusual topology resulting from the interactions of these two sequence elements may contribute to the regulation of GAP-43 expression. Supported by NIH grant EY07397.

503.16

CLONING OF THE HUMAN MYELIN P_2 PROTEIN GENE: ANALYSIS OF ITS STRUCTURE AND REGULATION. V. Narayanan and G. Tennekoon, Dept. of Neurology, The Johns Hopkins Univ. School of Medicine, Baltimore, MD.

Myelin P_2 protein, a 15 kDa cytosolic basic protein, is synthesized in Schwann cells and oligodendroglia. It belongs to a family of fatty acid binding proteins, and thus may have an important metabolic role during myelination. Our goal is to study the expression of this protein, investigate the nature of extracellular signals and intracellular second messengers that regulate this gene, and study the mechanisms of this genetic control.

We had previously characterized a cDNA clone encoding rabbit myelin P_2 protein (J. Biol. Chem. 263 8332 (1988)). We used this as a probe to screen a human genomic library constructed in λFIX^R (Stratagene Inc.) at low stringency. We identified two positive clones containing parts of the P_2 gene. One of these, HLF14.2.1, was partially sequenced and contains the first exon of this gene. The splice site between this exon and the first intron occurs at amino acid #24 (Gly), identical to the situation for the mouse myelin P_2 gene and others in this family of fatty acid binding proteins. We are studying the regulation of this gene in vitro by transfecting P_2 promoter-cat gene constructions into cultured Schwann cells and exposing them to various extracellular factors.

Supported by grants NS21700 and NS01282-02. V.N. is the recipient of a CIDA from the NINDS

503.17

MOLECULAR CLONING OF A cDNA ENCODING A POSTSYNAPTIC DENSITY GLYCOPROTEIN. B. Ni, J.W. Gurd and I.R. Brown. Departments of Zoology and Biochemistry, University of Toronto, Scarborough Campus, West Hill, Ontario, Canada M1C1A4.

We have raised a polyclonal antibody to the postsynaptic density glycoprotein PSD-gp180/65 which is concentrated in densities purified from the mammalian brain. In order to investigate the structure and function of PSD glycoproteins, Ab180/65 was used to screen a rat brain gt11 expression library and a positive clone NGB180/65 was isolated. Verification of the identity of this clone was obtained since i) fusion protein produced by NGB180/65 was recognized on immunoblots by Ab180/65 and ii) polyclonal antibodies raised against the fusion protein from NGB180/65 recognize PSD-gp180/65. A 350 bp cDNA insert from NGB180/65 detected two abundant mRNA species (1.8 and 3.8 kb) which are expressed at high levels in brain and at much lower levels in muscle. Interestingly, DNA sequence data for this 350 bp cDNA reveals substantial similarity but not identity with the cDNA sequence for calmodulin. Full length cDNA has been obtained by screening another brain cDNA library with the NGB180/65 insert. The restriction map of this cDNA is different from that known for calmodulin cDNAs. (Supported by grants from NSERC to I.B. and MRC to J.G.)

503.18

5' FLANKING SEQUENCES OF THE MOUSE 68 kDa NEUROFILAMENT GENE: ANALYSIS OF PROTEIN BINDING SITES. T. R. Ivanov and I. R. Brown. Department of Zoology, University of Toronto, Scarborough Campus, West Hill, Ontario, Canada, M1C 1A4.

Utilizing the enzyme DNase I, the chromatin conformation of cortical neurons has been recently analyzed (Neurochem. Res. 14:129-137, 1989). We now demonstrate the presence of 4 tissue-specific DNase I hypersensitive sites flanking the mouse 68 kDa neurofilament gene. One of these sites maps to a region near the TATA box while the other 3 sites are further upstream from the coding sequence. DNase I hypersensitive sites in non-neural systems are typically located near DNA regulatory elements to which nuclear proteins bind. To determine if the hypersensitive site situated near the TATA box is due to the presence of a specific protein-DNA complex, gel mobility shift assays were performed utilizing nuclear extracts and a labeled 228 bp DNA fragment. Several bands of reduced mobility were detected which can be competed away with excess cold 228 bp fragment. DNase I footprinting analysis is being performed to precisely determine the protein binding sequence. Analogous experiments are in progress for the remaining 3 hypersensitive sites. These studies may be useful in identifying the DNA sequences involved in the tissue-specific expression of the neurofilament gene. (Supported by grants from NSERC to I.B.)

530.19

STRESS ACTIVATION OF C-FOS EXPRESSION IN RAT BRAIN. H. Alho, J. Kononen*, J. Koistinaho* and A. Hervonen. Dept. of Biomedical Sciences and Dept. of Public Health, Univ. of Tampere, 33101 Tampere, Finland.

The proto-oncogene c-fos protein (Fos) is expressed in the nuclei of neurons in rodent brain. The basal expression of Fos is relatively low in most central nervous system regions and increases after generalized seizures. Wether the Fos can be included in response to synaptic activation by stress, rats were stimulated by immobilization and Fos expression was examined immunohistochemically.

Adult rats were immobilized for 1,2 and 3 h. Sections were stained for Fos by standard PAP-method. The primary antiserum was raised to a synthetic M-peptide region. The stimulated rats exhibited increased Fos immunoreactivity. This was most intense three hours after the immobilization. The major differences from control brains were the appearance of the Fos immunoreactivity in the paraventricular, habenular, arcuate, accumbens and septal nuclei. In cortex, the intensity of staining was increased in stimulated animals. The induction of Fos in adult neurons suggests that this protein may have role in CNS related to synaptic activation by stress.

530.20

CORRELATED INDUCTION OF C-FOS AND INCREASED [^{3}H]2-DEOXY-GLUCOSE UPTAKE DURING PILOCARPINE SEIZURES AND HARMALINE TREMOR IN MICE AND RATS. A. Hess and D. T. Hess*, Dept. of Anatomy, UMDNJ, R.W. Johnson Med. Sch., Piscataway, NJ 08854 and Dept. of Neurobiology, Stanford Univ. Sch. of Med., Stanford, CA 94305.

Previous evidence suggests that expression of some proto-oncogenes in adult neurons may be activity-dependent. 45 minutes after the onset of seizures produced by i.p. injection of 300mg/kg pilocarpine, c-fos protein (detected by immunohistochemistry) is increased substantially in neurons of the hippocampal dentate gyrus; uptake of [^{3}H]2-deoxy-glucose (2DG; detected by autoradiography) is also greatly increased within the dentate gyrus. Prior treatment with scopolamine (10mg/kg, s.c.) blocks these effects. 3 hours after the onset of tremor produced by i.p. injection of 15 mg/kg harmaline, c-fos protein (absent normally) is detected in neurons of the inferior olivary nucleus, and uptake of 2DG is greatly increased within the same region. After pilocarpine, c-fos induction and increased 2DG uptake are not detected in the substantia nigra, a possible site of seizure initiation, but occur in the secondarily affected dentate gyrus. After harmaline, c-fos induction and increased 2DG uptake occur in the inferior olive, the site of tremor initiation, but are not detected in the secondarily affected cerebellar cortex. Nonetheless, during pharmacologically induced seizure or tremor, increased neural activity as assessed by increased 2DG uptake is a concomitant of c-fos induction. Supported by NIH grant #21469

530.21

LIGAND AUTORADIOGRAPHIC RECEPTOR SCREENING: VALIDATION USING BETA-ADRENERGIC RECEPTOR cDNA. M. Rattray* S.L. Lautar* and G.R. Uhl2 (SPON: J.L. Rutkowski) NIDA/Addiction Research Center, Baltimore MD21224. & 2.Dept Neurol and Neurosci, Johns Hopkins University School of Medicine, Baltimore.

In order to validate a ligand-autoradiographic receptor screening (LARS) methodology, we have used hamster beta adrenergic receptor (ßAR) cDNA cloned into a eukaryotic expression vector (pßAR, a kind gift of Dr. R. Dixon). This plasmid contains sequences which allow replication and cDNA expression in permissive SV40 T-antigen expressing cell lines (i.e. COS, but not CV1 cells) We expressed foreign DNA in CV1 or COS cells, produced replicas of the transfected cells on polyester filters and identified ßAR expression by 125I-CYP receptor autoradiography. When pßAR was introduced into cells by spheroplast fusion, COS cell colonies with high levels of beta-receptor binding were observed on filter autoradiographs None were found on filter replicas of CV1 cells. Binding was displaced appropriately by propranolol and isoprotenerol. When pßAR-containing spheroplasts were mixed, in decreasing proportions, with control spheroplasts, we were able to detect positively-expressing colonies when as low as 0.01% of spheroplasts contained ßARcDNA. Furthermore, we have begun optimising DNA extraction procedures to recover Dpn-resistant plasmid DNA that has been replicated inside COS cells from small regions of the original cell culture plates which correspond to autoradiographic hotspots. These results indicate that the LARS technique may be useful for screening cDNA libraries for functional expression of ligand binding sites.

530.22

IDENTIFICATION OF LOCUS COERULEUS-SPECIFIC PROTEINS BY PROTEIN PHOSPHORYLATION AND SUBTRACTION HYBRIDIZATION. K. Saijoh*, R.S. Duman, and E.J. Nestler. Laboratory of Molecular Psychiatry, Departments of Psychiatry and Pharmacology, Yale University School of Medicine, New Haven, CT 06508.

Neurons of the locus coeruleus (LC), the major noradrenergic nucleus in brain, exhibit many distinct characteristics based on anatomical, electrophysiological, and pharmacological studies. Therefore, we have set out to identify proteins specific to the LC, and have done so by searching for phosphoproteins and mRNA species enriched in this brain region.

In one series of experiments, we have used protein phosphorylation and two-dimensional electrophoresis to detect LC-specific phosphoproteins. To date, we have identified 4 phosphoproteins that appear to be enriched in the LC, in that they are either not detectable, or present at much lower levels, in a large number of other brain regions studied. Of particular interest is an acidic, 62 kD protein, which is regulated in the LC by chronic in vivo morphine treatment.

In a second series of experiments, we have used molecular approaches to identify LC-specific mRNA species. Messenger RNA was isolated from LC and other regions of bovine brain, and the existence of a number of mRNA transcripts enriched in the LC was shown by in vitro translation. We have since constructed cDNA libraries of LC and of other brain regions using this mRNA, and will carry out subtraction hybridization to isolate and then clone LC-specific messages.

Identification of proteins expressed uniquely in the LC will provide important information concerning the molecular basis of some of the unique functional properties of LC neurons.

530.23

LIPOFECTION: A HIGHLY EFFICIENT PROCEDURE FOR THE TRANSIENT AND STABLE TRANSFECTION OF NAIVE AND NGF INDUCED PC12 CELLS. **Susanne R. Muller* and Stuart C. Feinstein (SPON: S. Fisher) Neuroscience Research Institute and Dept of Biological Sciences, Univ of Calif, Santa Barbara, CA. 93106**

Introduction of *in vitro* manipulated DNA into cultured cells is one of the most powerful molecular biology approaches to examine cell behavior. Unfortunately, a subset of cell lines transfect at very low efficiencies, thereby precluding many analyses. The NGF responsive cell line PC12, among the most widely studied cells in neurobiology, has been among these low efficiency lines. We have overcome this experimental impasse by adapting the "lipofection" procedure (Felgner et al. PNAS 84:7413). We have found that the cationic lipid "lipofectin", which forms unilamellar liposomes with DNA, introduces DNA into PC12 cells at extremely high efficiencies. Efficient transient transfection of PC12 cells, which has not been possible by other procedures, has been demonstrated by transfection of the plasmid RSV:CAT. This vector uses a Rous Sarcoma Virus LTR to direct transcription of a chloramphenicol acetyl transferase (CAT) reporter gene. Additionally, we have demonstrated that lipofectin can transfect NGF differentiated PC12 cells, allowing the transient introduction of genes into PC12 cells at any stage of their differentiation. Stable transfection, as assayed by resistance to the drug G418 (conferred by integration of the neo gene), occurs at a frequency of 3000 transfectants per 10^6 cells. This is 100 fold higher than obtained using the standard $CaPO_4$ procedure (Schweitzer et al. J. Cell Biol. 101:667). The availability of this technology will greatly increase the range of experimental manipulations possible in studying the mechanisms of NGF action and other issues in PC12 cells. (Supported by the Muscular Dystrophy Association and NIH Grant #RO1-NS24387)

530.24

CELLULAR AND MOLECULAR STUDIES OF HUMAN NEUROBLASTOMA CLUSTER CASES. M. Notohamiprodjo*, S.D. Styren, J. Rogers, and U. Rovigatti (SPON: M.L. Cheal). Institute for Biogerontology Research, Sun City, AZ 85351.

Neuroblastoma is believed to originate from the malignant transformation of neural crest progenitor cells which develop into the sympathetic nervous system, adrenal medulla, and adrenergic and cholinergic cells of the sympathetic chain and cranial ganglia. We have studied a group of human neuroblastoma cluster cases employing cellular and molecular techniques. Malignant cells were grown in vitro and oncogene alterations were analyzed by Southern and Northern blotting techniques as previously described (Rovigatti et al., Science, 232:398, 1986). Four neuroblastoma and two ganglioneuroma cases were identified as a cluster in Morgan City, Louisiana, because the diagnoses were linked in space and time. We studied the presence of N-myc amplification, which has been shown to correlate with diagnosis and prognosis in neuroblastoma. Although the two ganglioneuromas did not exhibit alterations, N-myc was amplifed in all four neuroblastomas, one of which showed both amplification and rearrangement. One of the tumors with N-myc amplification had a very high copy number (500-1000). Cultures from this tumor displayed very aggressive proliferative patterns and additional control sequences. Ultrafiltered (0.2 um) supernatants from these cultures induced morphologic transformation of control human and rat cells. The original neuroblastoma cells, supernatants, and transformed cells all exhibited presence of 70-100 nm diameter viral particles. Although 4-6 week old Fisher 341 rats did not show overt pathology when infected with the supernatants, our preliminary data suggest that litters from infected mothers develop a very rapid, lethal disease that resembles neuroblastoma. This model system may be useful for investigating the etiology of neuroblastoma and the origin of neuroblastoma cluster cases.

504.1

COEXPRESSION OF OXYTOCIN AND VASOPRESSIN IN HYPOTHALAMIC NEURONS OF PARTURIENT MICE. G.F. Jirikowski, J.F.Ramalho-Ortigao, K.W. Kesser, J.D. Caldwell. Abt. Anatomie und Zellbiologie, Sektion Polymere and Abt. Anatomie, University of Ulm, FRG and Dept. of Psychiatry, University of North Carolina, Chapel Hill.

The hypothalamic neuropeptides vasopressin (VP) and oxytocin (OT) are known to be synthesized in distinct populations of neurons in the classical magnocellular nuclei. The synthetic and secretory activity of magnocellular hypothalamic neurons is influenced by steroid hormones. In the present study we observed changes in expression of these peptides in parturient mice. Parturient mice or mice at proestrus (controls) were killed by cardiac perfusion. Tissue samples of the magnocellular nuclei and the pituitary were embedded in Epon. OT and VP immunocytochemistry was performed on consecutive semithin sections . Alternating sections were hybridized in situ with synthetic oligonucleotide probes, complementary either to OT or to VP m-RNA. Probes labelled with 5-bromo 2-deoxyuridine (BrdU) were detected immuncytochemically. While in control animals OT- and VP immunostaining and hybridization with the corresponding probe was always found in distinct populations of neurons, coexistence of the two peptides and the respective m-RNA sequences could be found in paraventricular and supraoptic neurons of parturient mice. In the posterior lobe of controls OT and VP immunostaing was found in distinct Herring bodies whereas coexistence of the two peptides was found in numerous nerve endings of parturient mice.

The present results indicate a transient coexpression of VP and OT in hypothalamic neurons of parturient mice. The functional significance could be seen in the high demand of hormonal OT at parturition. It is likely that the observed shift in peptidergic specificity is mediated through genomic action of ovarian steroid hormones.

504.2

DEVELOPMENTAL REGULATION OF VASOACTIVE INTESTINAL PEPTIDE-VIP mRNA IN DEVELOPING SPINAL CORD. D.V. Agoston* I. Gozes, L.E. Eiden* and D.E. Brenneman (SPON: J. Gershoni). Lab. of Cell Biology, Mol. Genetics and Dev. Neurobiol. NIMH and NICHD, Bethesda, MD 20892

Previous studies have shown that electrical activity and vasoactive intestinal peptide influence the survival of developing spinal cord neurons in dissociated cell cultures. A critical period for neuronal cell death begins approximately on day 7, a time when all spinal cord neurons exhibit spontaneous action potentials. We now analyze the time course for VIP-gene expression in these cultures and also examine the effect of electrical blockade on this expression. Quantitative Northern blot hybridizations have been done with high specific activity labeled VIP-riboprobes. Our results show that the VIP-mRNA (about 2000 bases long) appeared three days after plating of mixed cultures of spinal cord and dorsal root ganglia (DRG) cells, exhibiting a peak at 9-12 days post plating and decreasing thereafter. This time course differs from that of the actin-mRNA which codes for a structural protein and peaks at 15 days in culture, maintaining a plateau thereafter. Blockade of electrical activity with tetrodotoxin had no effect on the VIP-mRNA levels. Taken together, our results indicate that VIP-mRNA is elevated during a critical period of neuronal development; moreover, in these cell cultures VIP-gene expression is probably not regulated by electrical activity.

(D.V.A. is supported by the Deutsche Forschungsgemeinschaft, I.G. is on Sabbatical from the Weizmann Inst. of Science).

504.3

CORRELATED CHANGES IN HYPOTHALAMIC VASOPRESSIN (VP) MRNA AND PLASMA VP LEVELS DURING THE DEVELOPMENT OF DOC-SALT HYPERTENSION. R.D.Hartman, D. Ashen*, J.M. Hamlyn*, S. L. Petersen and C.A.Barraclough, Dept. of Physiol., Univ. Maryland, Sch. of Med., Baltimore, MD 21201.

The deoxycorticosterone (DOC)-salt model of hypertension has been shown to be VP-dependent. In these studies, we investigated whether levels of VP mRNA in the hypothalamic paraventricular (PVN) and supraoptic nuclei (SON) change during the development of DOC-salt hypertension. Male Sprague-Dawley rats (80-90 gm) were unilateral nephrectomized (day -14) and 7 days later (day -7) their drinking water was replaced with 0.9% saline. A week later (day 0), Silastic capsules containing DOC (100 mg/kg in sesame oil) or sesame oil alone (sham) were implanted sc. On days 5, 12 and 30, 24 hr urines were collected, mean arterial pressure (MAP) was measured, animals were sacrificed and trunk blood was collected. VP in urine and plasma was measured by RIA. Brains were stored frozen (-70 C) and later sectioned (12 u) on a cryostat. In situ hybridization was performed on brain sections using an oligodeoxynucleotide probe complementary to VP mRNA (Lightman & Young, J. Physiol. 394:23, 1987) and labelled with [35S]-dATP. Following hybridization, sections were placed against autoradiographic film. The relative density and area of each nucleus was quantified using a Bioquant IV Image Analysis System. DOC treatment increased MAP (days 5,12,30), urinary VP output (days 5,12,30) and plasma VP (day 30). VP mRNA levels (density) in the PVN were elevated 8.6% and 16.5% on days 12 and 30, respectively, in DOC-treated rats compared to shams. No significant changes in VP mRNA were observed in SON. Thus, VP neurons in PVN, possibly a subpopulation which projects to cardiovascular regulatory centers, may be important in the onset of DOC-salt hypertension.

504.4

AGE-RELATED DECLINE OF VASOPRESSIN GENE EXPRESSION IN NEURONS OF THE BED NUCLEUS OF THE STRIA TERMINALIS. D. J. Dobie*, M. A. Miller*, J. H. Urban and D. M. Dorsa, (SPON: M. Raskind), Dept. Psych/Behav. Sci., Univ. of WA., Seattle, WA 98195; GRECC, VA Med Ctr, Seattle, WA 98108

Vasopressin (VP) immunoreactivity in the bed nucleus of the stria terminalis (BNST) decreases in aged rats. Plasma testosterone (T) levels also decline with age. We have previously reported that the biosynthetic capacity of BNST VP neurons is regulated by T. To determine whether aging influences VP mRNA in the BNST, we used in situ hybridization to measure VP mRNA levels in 3-month (n=4), 14-month (n=4), and 24-month old (n=5) Fischer 344 male rats.

Fewer labeled cells were observed in the BNST of 24-month animals than either the 3-month ($p<0.01$) or the 14-month ($p<0.05$) animals (X±SEM: 77.5±6.5, 3-month; 67.8 ±4.8, 14-month; 35.4±11.2, 24-month). Plasma T levels were also reduced in 24-month rats compared to 3-month ($p<0.01$) or 14-month ($p<0.05$) rats (X±SEM: 1.1±0.2ng/ml, 3-month; 0.9±0.3ng/ml, 14-month; 0.2±0.05 ng/ml, 24-month). Estradiol levels did not differ significantly.

These results indicate that the biosynthetic capacity of VP neurons in the BNST decreases with age, possibly due to a decline of circulating T.

504.5

DEXAMETHASONE DECREASES VASOPRESSIN mRNA EXPRESSION IN CELLS OF THE BED NUCLEUS OF THE STRIA TERMINALIS AND MEDIAL AMYGDALA. J. H. Urban, M. A. Miller,* and D. M. Dorsa. GRECC, VA Medical Center, Seattle, WA 98108

Expression of vasopressin (VP) mRNA in the paraventricular nucleus is influenced by glucocorticoids. To determine whether VP cells in the bed nucleus of the stria terminalis (BNST) and medial amygdala (AME) respond to changes in glucocorticoid levels, in situ hybridization and quantitative autoradiography were used to measure VP mRNA in the BNST and AME of rats that were sham-operated (SH), adrenalectomized (ADX; 14d) and dexamethasone replaced (DEX; 75µg/100g BW; 14d). ADX did not produce a significant increase in the number of labeled cells in the BNST (SH 75±4; ADX 85±13) or AME (SH 68±4; ADX 63±4), or grains/cell in the BNST (SH 54.2±1.8; ADX 60.0±4.9) or AME (SH 65.1±2.3; ADX 70.6±3.2). Treatment with DEX decreased the number of cells observed in the BNST (51.6± 5.0; $p<0.05$) and AME (50.8±2.8; $p<0.05$) when compared with ADX. Grains/cell were significantly reduced in the BNST (41.4±2.2; $p<0.05$) from both SH and ADX groups, and in the AME (59.0±2.1; $p<0.05$) from ADX. Plasma testosterone (T) levels were decreased in the DEX group (SH 2.92±0.21; ADX 2.56±0.50; DEX 0.73±0.17ng/ml; $p<0.01$). These results suggest that ADX is not a potent stimulus in altering VP activity in the BNST and AME, and that DEX treatment reduces VP gene expression in these neurons possibly by altering plasma T levels.

504.6

TESTOSTERONE REGULATION OF VASOPRESSIN mRNA IN THE BED NUCLEUS OF THE STRIA TERMINALIS IS DOSE DEPENDENT. M. A. Miller,* J. H. Urban, and D. M. Dorsa (SPON: C. Wilkinson). GRECC, VA MEDICAL CENTER, SEATTLE, WA 98108.

We have previously reported that castration of adult male rats results in the disappearance of cells expressing vasopressin (VP) mRNA throughout the bed nucleus of the stria terminalis (BNST). To determine whether testosterone (T) regulates the expression of the VP gene in a dose dependent way, we have used in situ hybridization to measure VP mRNA in neurons in the BNST of intact (n= 5: plasma T=2.6±1.4ng/ml), castrated male rats treated with low levels of T (n=5: plasma T=1.2±0.9 ng/ml), and castrated male rates with high levels of T (n=5: plasma T=7.6±0.7ng/ml). The number of labeled cells (unilateral) and the average number of grains/cell were compared in four sections through the BNST.

Treatment of castrated rats with high levels of T increased both the number of cells ($p<0.05$) and the average intensity of labeling ($p<0.05$) over both the low T replaced and intact levels. Rats treated with high T averaged 102±10 cells compared to 78±5 cells in the low T group and 72±3 cells in the intact group. Average grains/cell was 90±4 in the high T group versus 70±4 and 75±2 for the low T and intact groups, respectively. We are currently assessing the time course and the mechanism of T modulation of VP gene expression.

504.7

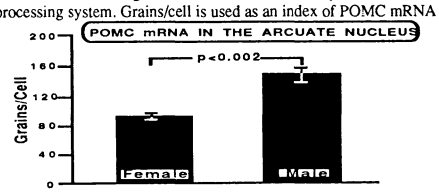
GLUCOCORTICOID REGULATION OF PREPROENKEPHALIN mRNA IN THE RAT STRIATUM AS DETERMINED BY IN SITU HYBRIDIZATION. H.M.Chao and B.S.McEwen. Lab. of Neuroendocrinology, Rockefeller Univ., N.Y.,N.Y. 10021.

Our previous studies have shown that in the striatum, the expression of preproenkephalin (ppE) mRNA is regulated by glucocorticoids. There is a decrease in striatal ppE mRNA after adrenalectomy (ADX) and ADX animals replaced with corticosterone express higher levels of striatal ppE mRNA than ADX animals. In this study we have used in situ hybridization to assess the level of ppE mRNA expression. We determined that the increase in striatal ppE mRNA is evident after 16 hours, but not after 2 hours, of corticosterone replacement of ADX animals. The effect of corticosterone treatment on ppE mRNA expression in ADX rats is not mimicked by the increase in endogenous corticosterone produced by acute stress in adrenally intact animals. We are currently investigating the effects of chronic stress and of the diurnal variation in endogenous glucocorticoid levels on ppE mRNA expression.(Supported by MH41256 and NS07080.)

504.8

SEXUAL DIMORPHISM OF PRO-OPIOMELANOCORTIN GENE EXPRESSION IN THE ARCUATE NUCLEUS OF THE RAT BRAIN. J.A. Chowen-Breed*, J. Argente*, R.A. Steiner and D.K. Clifton*, (SPON: R. Caudle) Depts.of Physiol. & Biophys. and Ob. & Gyn., U. of Wash., Seattle, WA 98195.

Sexually differentiated secretory patterns of pituitary hormones are thought to be orchestrated by a steroid-sensitive neural substrate, which becomes organized during a critical period in neonatal life. ß-Endorphin, derived from the precursor molecule POMC, has been implicated in the regulation of anterior pituitary function. We sought evidence for sexual dimorphism in POMC gene expression in cells of the arcuate nucleus by comparing POMC mRNA content within individual neurons between male (n = 3) and proestrous female (n = 3) rats. Animals were sacrificed and brain slices through the arcuate were prepared for *in situ* hybridization with an RNA probe for the POMC message. Individual cells were analyzed with a computerized image processing system. Grains/cell is used as an index of POMC mRNA content.



POMC mRNA content in neurons of the rostral arcuate was significantly greater in males compared with proestrous females. This difference may be attributable to either the unique genetic elements or the different hormonal milieu of the sexes.We deduce that the sexually dimorphic patterns of anterior pituitary hormone secretion could, in part, be attributed to this sex difference in the activity of ß-endorphin-secreting cells.

504.9

EXPRESSION OF HYPOTHALAMIC NEUROPEPTIDES IN FOOD-RESTRICTED RATS. L. S. Brady, M. A. Smith, P. W. Gold and M. Herkenham. Unit on Functional Neuroanatomy, Clinical Neuroendocrinology Branch, NIMH, Bethesda, MD 20892.

Disturbances in CNS peptide systems are evident in patients with eating disorders such as anorexia nervosa. We examined mRNA expression of hypothalamic peptides in food-restricted rats to characterize the consequences of weight loss. Food intake was restricted (10 g/day) in male and female Sprague-Dawley rats for two weeks; animals lost 30% of their body weights. Cryostat-cut sections through the hypothalamus were hybridized with ^{35}S-oligonucleotide probes for pro-opiomelanocortin (POMC), neuropeptide Y (NPY), corticotropin releasing hormone (CRH) and other peptides. Sections were exposed to film for quantitative autoradiography and then dipped in nuclear emulsion. POMC message was reduced in the arcuate nucleus by 43-47% relative to the control group; this change represents a 26-36% decrease in the number of labeled cells and a 59-66% decrease in grains/cell. NPY message was increased in the arcuate nucleus by 30-58%; the number of labeled cells was increased 70-155% and grains/cell was increased 117-150%. CRH, NPY, dynorphin and vasopressin mRNAs were not altered in the paraventricular nucleus. These results suggest that decreased β-endorphin and increased NPY concentrations in the CSF of anorexic patients reflect normal physiological consequences of weight loss. Increased secretion of CRH and other peptides in the CSF of anorexics may reflect pathological consequences of the disease.

504.10

REGULATION OF PREPROENKEPHALIN mRNA LEVELS IN CULTURED RAT ASTROCYTES. D.K. Batter, M.-H. Vilijn, and J.A. Kessler. Departments of Neurology and Neuroscience, Albert Einstein College of Medicine, Bronx, New York 10461.

Previous work from this laboratory has demonstrated the expression of preproenkephalin (PPE) mRNA in cultured astrocytes. PPE mRNA was found in both embryonic and neonatal astrocytes isolated from five different brain areas, with levels (per μg total RNA) showing regional heterogeneity (Vilijn, M.-H., Vaysse, P. J.-J., Zukin, R.S., and Kessler, J.A., Proc. Natl. Acad. Sci. 85:6551, 1988). Here we show that astroglial preproenkephalin mRNA expression is regulated, at least in part, through a cAMP second messenger system. Confluent cultures of dissociated neonatal hypothalamic astrocytes were treated with the β-adrenergic agonist isoproterenol (100 nM) for varying times (2,3,4, and 16 hrs.). Northern blots containing total cellular RNA were hybridized with a riboprobe for preproenkephalin mRNA. PPE mRNA levels in treated cultures increased 3-5 fold over controls by 16 hr. The isoproterenol-stimulated increase could be blocked almost entirely by treatment with the β-antagonist propranolol. This increase could be mimicked by treatment with 5 μM forskolin. These data strongly suggest that astroglial PPE mRNA can be stimulated by a cAMP transduction system, and that this system can be activated through β-adrenergic receptors. Levels of PPE mRNA were also stimulated by treatment of cultured astrocytes with 100 μM cytosine arabinoside, raising the possibility that PPE mRNA metabolism may be related to the cell cycle. In toto, these observations suggest that levels of preproenkephalin mRNA can be regulated by several different physiological stimuli.

504.11

EVIDENCE THAT NEUROENDOCRINE SUBSTANCES CAN INFLUENCE IMMUNOLOGICALLY IMPORTANT GENES AT THE TRANSCRIPTIONAL LEVEL. H. Kulaga*+, J.A. Sogn, A.J. Adams+ and T.J. Kindt. +Neuropsychiatry Branch, NIMH, Washington, D.C. 20032 and LIG, NIAID, Bethesda, Maryland 20892.

Agents important to neuroendocrine function were incubated with a rabbit T-cell line, RL-5. Cells were exposed to ACTH, substance P, somatostatin, ß-endorphin and leu enkephalin for 24 hr at concentrations which did not affect cell viability or overall cell cycling as assessed by trypan blue exclusion and ^{3}H-thymidine incorporation. Genomic and cDNA clones corresponding to Major Histocompatability Complex Class I, Class II, T-cell receptor α and β chain genes were then used to screen RNAs isolated from pretreated cultures. Striking variations in the level of expression of these genes were shown by Northern blot analysis. Alterations in both levels and length of hybridizing species were particularly pronounced in preparations obtained from ACTH treated cells. Transcription of constituatively expressed structural genes (α actin, histone 4B) was also measured. The results of these experiments indicate that neuroendocrine modulators, which have previously been shown to alter immunocompetence, can exert a direct effect at the nucleic acid level.

504.12

CORTICOTROPIN-RELEASING HORMONE: PRE- AND POSTNATAL GENE EXPRESSION IN THE DEVELOPING RAT BRAIN.
T. Z. Baram MD, PhD and L. Schultz. Dept Neurology, USC and div. Neurology, CHLA, Los Angeles, CA 90054.

Corticotropin- Releasing Hormone (CRH), is a neuropeptide involved in hormonal and behavioral stress responses in mammals. The brain- adrenal axis and the steroid response to stress are depressed in the perinatal period, possibly protecting the brain from high, toxic steroid levels generated by the stress of birth. The role of CRH in the perinatal stress response, and the peptide's expression in hypothalamic and extra- hypothalamic sites during that critical period have not been elucidated. We have studied the ontogeny and regulation of CRH gene expression in rat brain, and especially in the paraventricular nucleus (PVN) at both mRNA and peptide levels. We utilized fetal rats (days: 16, 18, 20) and postnatal rats, 1, 4, 7, 10, 14, 29 and 25 days old. CRH mRNA is localized by in situ hybridization using an S^{35}-labelled synthetic 60-mer oligonucleotide probe, with a sense strand control. Message is first detectable on the 18th fetal day in the PVN, which is compatible with the formation of the parvocellular cells on the 17th fetal day (Altman, J and Bayer, SA: Adv. Anat. Emb. Cell Biol. 100, 1-178; 1986). Probe signal is also increased on the 18th fetal day in the ventral septal region. Message decreases perinatally in both regions, concurrent with the non-responsive period of the CRH-adrenal axis, suggesting that a decrease in CRH gene expression may contribute to this phenomenon.

504.13

EFFECTS OF CHRONIC ANTIPSYCHOTIC DRUGS ON PREPROTACHYKININ mRNA IN LIMBIC AREAS OF RAT BRAIN. K.Shibata, D.M.Haverstick and M.J.Bannon. Center for Cell Biology, Sinai Hosp. of Detroit and Cellular and Clinical Neurobiology Program, Wayne State Univ., Detroit, MI 48235

While tachykinin biosynthesis in striatonigral neurons has been intensively studied, very little is known about the regulation of tachykinin gene expression in limbic nuclei. The present experiments used nuclease-RNA protection techniques to determine the basal level of the preprotachykinin (PPT) A gene mRNA in various limbic nuclei and the effects of chronic antipsychotic drug (APD) treatment. The rank order of PPT mRNA content was: striatum $\geq$ nucleus accumbens (NA) > septum, amygdala > bed nucleus of stria terminalis (BST). In all brain regions, the proportions of the various PPT mRNAs derived by alternate RNA splicing was similar. After chronic injection of the prototypical APD haloperidol (1mg/kg/d x 8 d), PPT mRNAs were significantly decreased in the striatum but less affected in limbic nuclei. In contrast, the atypical APDs clozapine (20mg/kg/d/ x 8d) and l-sulpiride (35mg/kg/d x 8d) did not decrease striatal PPT mRNAs but did alter PPT mRNAs of NA and BST. In all drug experiments, there was no drug-induced alternation in PPT mRNA splicing. Extension of these studies should help to clarify whether or not altered PPT gene expression could be involved in the clinical response to APD.

504.14

DEVELOPMENTAL PROFILE OF PREPROTACHYKININ mRNAs IN RAT STRIATUM. D.M.Haverstick, M.Jeziorski and M.J.Bannon. Center for Cell Biology, Sinai Hospital of Detroit and the Cellular and Clinical Neurobiology Program, Wayne State University, Detroit, MI 48235.

Although the adult basal ganglia is the brain region with the highest content of the peptide substance P (SP), the development of this system has not been quantitatively analyzed. The preprotachykinin (PPT) A gene consists of 7 exons encoding the tachykinin peptides SP, neurokinin A (substance K) and several N-terminally extended forms of neurokinin A. Multiple PPT mRNAs arising from alternate PPT primary transcript splicing encode different combina-tions of these peptides. Since some other neurally expressed gene transcripts are alternately spliced in a developmentally specific manner, both the absolute amount and proportion of the various PPT mRNAs in rat striatal tissue (ranging from embryonic day 19 to adult animals) was quantitated using a T1 nuclease/RNA protection protocol. PPT mRNA levels at E-19 (2 days after SP is detected using histochemistry) were approximately 20% of adult levels, which were reached during the third postnatal week. During the entire developmental time course, the proportion of the various PPT mRNAs was unchanged. These and other data suggest that striatal tachykinin neurons are committed to the production of an invariant ratio of the various tachykinin peptides.

504.15

DISTRIBUTION OF NEUROTENSIN/NEUROMEDIN N mRNA IN RAT FOREBRAIN: UNEXPECTED ABUNDANCE IN HIPPOCAMPUS AND SUBICULUM. M.J. Alexander, M.A. Miller, D.M. Dorsa, B.P. Bullock*, R.H. Melloni, Jr.*, P.R. Dobner*, and S.E. Leeman, Univ. of Mass. Med. Ctr., Worcester, MA 01655, and V.A. Med. Ctr., Seattle, WA 98108.

We have used *in situ* hybridization with a ^{35}S-labeled antisense RNA probe to determine the regional distribution of mRNA encoding the neurotensin/neuromedin N (NT/N) precursor in the forebrain of the adult male rat. Specificity of labeling was confirmed with a sense RNA probe and competitive displacement of the labeled antisense probe by excess unlabeled antisense RNA. Cells containing NT/N mRNA are widely distributed in the forebrain. These areas include the septum, accumbens nucleus, diagonal band of Broca, bed nucleus of the stria terminalis, preoptic area, hypothalamus, amygdala, caudate-putamen, and piriform and retrosplenial cortex. In general, the regional distribution of NT/N mRNA corresponds to the previously determined distribution of NT-immunoreactive cell bodies; however, several notable exceptions were observed. The most striking difference occurs specifically in the CA1 region of the hippocampus, where intense labeling is associated with the pyramidal cell layer despite the reported absence of NT-immunoreactive cells in this region. Analysis of microdissected tissue by S1 nuclease protection assay confirmed the abundance of authentic NT/N mRNA in CA1. A second major discrepancy between NT/N mRNA abundance and NT immunoreactivity occurs in the intensely labeled subiculum, a region that contains only scattered NT-immunoreactive cells in the adult. Although these discrepancies may reflect translational regulation, our results raise the possibility that, in specific regions of the forebrain, NT/N precursor is processed to yield products other than NT. In addition, these results provide an anatomical basis for studying the regulation of NT/N mRNA levels in the forebrain.

504.16

THE ACUTE EFFECTS OF HALOPERIDOL ON NEUROTENSIN mRNA IN RAT STRIATUM AS DETERMINED BY FLUORESCENCE *IN SITU* HYBRIDIZATION. F. G. Williams*, M.P. Murtaugh* and A.J. Beitz (Spon: D. Brown) Dept. of Veterinary Biology and Veterinary Pathobiology, College of Veterinary Medicine, Univ. of Minn., St. Paul, MN 55108.

The neuroleptic haloperidol (HAL) has been shown to increase levels of neurotensin (NT) in the rat striatum. Immunohistochemistry of NT-containing neurons have localized the HAL effect to cells in the caudate-putamen, nucleus accumbens, and olfactory tubercle. The present study employed *in situ* hybridization to examine the effects of HAL on the cellular distribution and levels of neurotensin/neuromedin-N mRNA (NT-mRNA).
Male Sprague-Dawley rats were given 2mg/kg HAL (ip.) or vehicle alone at 17 and 10 hours before sacrifice. A 45 base oligonucleotide complementary to the peptide coding sequence of rat NT- mRNA was biotinylated using bio-11-dUTP and terminal transferase and purified by spun column chromatography through Sephadex G-10. Five or 10 ng of the labeled oligo was hybridized to cryosections (15μ) of fresh-frozen rat brain in 25 μl standard hybridization medium. After two 5min. rinses in 4X SSC/50% Formamide (37 C) and a 10 min. rinse in 2X SSC (room temp.). FITC-avidin DN (Vector Labs) diluted 50:1 in bicarbonate buffer pH 8 was applied to the tissue for 1 hour at room temperature, followed by three 10 minute washes in 2X SSC. Fluorescent perikarya in the striatum and midbrain were mapped using the rat brain atlas of Paxinos & Watson. Haloperidol caused major modifications in the number and distribution of neurons containing NT-mRNA. The greatest increase in hybridized perikarya occurred in the ventral accumbens and caudate/putamen. Significant increases in hybridized neurons were also observed more medially in the septal nuclei and the medial/ventral shell of accumbens. Computerized pseudocolor maps were made to compare the hybridization fluoresence intensities of normal and HAL-treated ventral striatal neurons. The maximal perikaryal message levels were not increased by HAL. Rather, the frequency of hybridization-positive neurons was increased by HAL. These studies indicate that HAL induces an increase in the number of NT-mRNA containing neurons in several areas and further suggests that the tonic inhibitory effects of dopamine on NT biosynthesis are not limited to the striatum. Supported by NIH grants DE06682 & NS19208

504.17

ANALYSIS OF A BRAIN-SPECIFIC mRNA AND ITS ENCODED SECRETOGRANIN-LIKE POLYPEPTIDE. H.-P. Ottiger, E.F. Battenberg, A.-P. Tsou*, F.E. Bloom, and J.G. Sutcliffe.(SPON:K.A.Nave) Research Institute of Scripps Clinic, La Jolla, CA 92037.

The rat 1B1075 mRNA encodes a 533-residue novel, secretogranin-like acidic protein with an apparent secretion signal, several pairs of tandem basic residues, and internally repeated sequence elements. 1B1075 transcripts are detected by blotting and in situ hybridization at highest levels in frontal and parietal cortex, the bed nucleus of the stria terminalis and pituitary corticotrophs, at lower levels in most other brain regions, but in none of several other tissues. Utilizing antisera to synthetic peptide fragments of the predicted protein sequence, we detect a brain- and pituitary-specific 57 kDa acidic protein that in immunohistochemistry experiments is found in cellular processes and fiber tracts, generally consistent with axonal transport from the cell bodies identified by in situ hybridization. Although the function of the 1B1075 protein is presently unknown, a mouse ablated for the 1B1075-homologous gene has been produced, that allows further studies of the potential involvement of this molecule in the secretory pathway.

504.18

INCREASED HYPOTHALAMIC PREPRONEUROPEPTIDE Y mRNA CONTENT FOLLOWING FOOD DEPRIVATION. J.D. White and M. Kershaw* (SPON: P. Camp). Div. Endocrinology, Dept. of Medicine and Dept. of Neurobiology and Behavior, SUNY, Stony Brook, NY 11794

Neuropeptide Y (NPY) potently stimulates food intake in rats by acting within the hypothalamic paraventricular nucleus and the content of NPY within the paraventricular nucleus has been shown to respond to food deprivation and re-feeding. In this study we examined the possibility that NPY gene expression is increased following food deprivation by measuring hypothalamic content of preproNPY mRNA. Adult male Sprague-Dawley rats were allowed free access to water but were subjected to either overnight or 72 hours of food withdrawl, or not. Total hypothalamic RNA was isolated using a guanadine isothiocyanate/ phenol-chloroform extraction protocol. The content of preproNPY mRNA was determined by solution hybridization of total RNA to ^{32}P-cRNA probe and RNase protection-urea/PAGE analysis. This study revealed an approximate 2-fold increase in hypothalamic preproNPY mRNA in overnight fasted vs. control rats and an approximate 4-fold increase in 72 hour food deprived vs. control rats. To determine the cellular localization and the specificity of the increase in hypothalamic preproNPY mRNA content, in situ hybridization analysis was used. Ketamine/xylazine anesthesized control or 72 hr food deprived rats were perfused with paraformaldehyde and free-floating 30 μm coronal sections through the hypothalamus were allowed to hybridize with ^{35}S-cRNA probe. The densitiy of hybridization was assessed using image analysis of film autoradiograms and microscopic analysis of emulsion coated sections. This study revealed an increase in hybridization in the arcuate nucleus of the hypothalamus of similar magnitude to that observed using nuclease protection with no change observed over neocortex or reticular nucleus of the thalamus. These data are consistent with the hypothesis that expression of hypothalamic NPY is modulated by peripheral metabolic status. (Supported by NIH MH42074)

504.19

PREPRONEUROPEPTIDE Y mRNA CONTENT IS SPECIFICALLY INCREASED IN HYPOTHALAMUS OF OBESE ZUCKER FATTY RAT. G. Sanacora*, M. Kershaw* & J.D. White (SPON: M. Berelowitz), Div. Endocrinology, Dept. Medicine, SUNY, Stony Brook, NY 11794

Neuropeptide Y (NPY) is a 36 amino acid peptide that potently stimulates carbohydrate feeding when injected into the hypothalamic paraventricular nucleus in rats and when given chronically can lead to hyperphagia and obesity. NPY immunoreactive cell bodies have been localized to the arcuate nucleus and project to the paraventricular nucleus. In this study, we investigated the possibility that preproNPY mRNA levels are increased in the arcuate nucleus of obese Zucker "fatty" rats compared to their lean littermate controls. Total RNA was isolated from whole hypothalamic dissections from obese and lean Zucker male and female rats. PreproNPY mRNA content was measured using RNase protection analysis followed by urea/ PAGE and quantitation of autoradiographic bands using 2-D laser densitometry. This analysis revealed an approximate 3-fold increase in hypothalamic preproNPY mRNA levels in samples from obese male and female rats. To assess the specificity of this increase in hypothalamic preproNPY mRNA levels, preproNPY mRNA levels were measured in eight additional brain regions (cortex, olfactory bulb, cerebellum, hippocampus, brain stem, striatum, thalamus and lateral geniculate). Preliminary results suggest that the increase in preproNPY mRNA levels is specific to the hypothalamus. In situ hybridization analysis confirmed that the increase in hypothalamic preproNPY mRNA content was localized to the arcuate nucleus. These data are consistent with the hypothesis that regulation of hypothalamic NPY expression is disturbed in obese Zucker rats and that this disturbed regulation may play a role in the etiology of obesity in these animals. (NIH MH 42074 & SUNY Faculty Development Award).

504.20

STIMULATION OF HIPPOCAMPAL PREPRONEUROPEPTIDE Y EXPRESSION FOLLOWING RECURRENT SEIZURE. G.L. Yount*, C.M. Gall[1] and J.D. White, Div. Endocrinology, Dept. Medicine and Dept. Neurobiology and Behavior, SUNY, Stony Brook, NY 11794 and [1] Dept. Anatomy and Neurobiology, Univ. Cal., Irvine, CA 92717

Previous studies have shown that limbic seizures, induced by a unilateral focal electrolytic lesion of the hippocampal dentate gyrus hilus, lead to dramatically increased synthesis of enkephalin by dentate gyrus granule cells and neurons in entorhinal cortex. In the present study, the influence of hilus lesion (HL)-induced seizures on the abundance and distribution of mRNA coding for preproneuropeptide Y (ppNPY) in rat hippocampus was analyzed. By nuclease protection analysis, a large increase in ppNPY mRNA content was observed following HL-seizures: 3-fold by 6 hrs post-HL that reached a maximum (50-fold) by 18 hrs post-HL and returned to control values by 4-10 days post-HL. By *in situ* hybridization analysis, greatest densities of labeled neurons in control rats were seen in dentate gyrus hilus and surrounding stratum pyramidale. Dentate gyrus granule cells were only very rarely seen to be labeled. By 6 hrs post-HL the distribution and number of labeled hippocampal neurons appeared normal with an increase in the density of hybridization associated with individual neurons. At later times, hybridization over dentate gyrus granule cells (10 hr post-HL) and CA1 pyramidal cells (17 hr post-HL) became evident such that by 24 hrs post-HL, hybridization was elevated far above normal over stratum granulosum, CA1 stratum pyramidale and superficial entorhinal cortex. By 2 days post-HL, the granule cells were no longer labeled whereas hybridization remained elevated in CA1 stratum pyramidale and entorhinal cortex. By 4 days post-HL, hybridization appeared normal in all fields. These data demonstrate that seizures induce a transient increase in ppNPY expression in several populations of hippocampal neurons including cells not previously considered to contain this neuropeptide. (MH 42074 [JDW] & NS26748 [CMG])

UPTAKE, STORAGE, SECRETION AND METABOLISM IV

505.1

DIRECT UPTAKE AND RELEASE OF 3H-N-ACETYLASPARTYLGLUTAMATE FROM CHICK RETINAL NEURONS. J.H. Neale and L.C. Williamson. Dept. Biology, Georgetown Univ., Washington D.C. 20057

Data on the neuronal localization and synaptic release of N-acetylaspartylglutamate (NAAG) has stimulated interest in its extracellular fate, particularly in retinal tissue where we have demonstrated neuronal release of NAAG.

Chick retinas were incubated in vitro with 3H-NAAG in order to assay both extracellular peptidase activity against it and direct transport of the peptide into retinal cells. The transport of 3H-NAAG was distinguished from transport of peptidase-released 3H-glutamate by incubation of retinal tissue with a series of peptidase inhibitors. Time-dependent 3H-NAAG uptake into retinal cells was observed. The intracellular appearance of 3H-NAAG was increased by inhibition of extracellular peptidase activity against 3H-NAAG, a likely result of greater availability of the peptide for transport. To explore the possibility that direct NAAG uptake may be coupled to a releasable synaptic pool in these cells, retinas were incubated with a series of physiological buffers to stimulate release after a 30 min incubation with 3H-NAAG. 3H-NAAG incorporated into retinal cells by direct uptake was released upon depolarization and this release process required extracellular calcium.

505.2

EFFECTS OF TAURINE ON PROTEIN PHOSPHORYLATION IN CORTICAL SYNAPTOSOMES OF RATS. Y.-P. Li* and J.B. Lombardini (SPON: W.H. Lyness). Texas Tech Univ. HSC, Lubbock, TX 79430.

Taurine (TAU), an amino sulfonic acid occurring free in excitable tissues, has been proposed to be either a neurotransmitter or neuromodulator in the CNS. However, its function and mechanism of action are not known. In these studies we report on the inhibitory effects of TAU on the in vitro phosphorylation of specific proteins found in an osmotically shocked synaptosomal preparation of the rat cortex. SDS-PAGE demonstrated that the phosphorylation of proteins with molecular weights of 140K and 20K was inhibited by physiologic TAU concentration (10 mM). Higher TAU concentrations (20 mM) had a general inhibitory effect on total protein phosphorylation. Time studies indicated that the TAU effect was observed after an incubation of 1 minute; increasing the incubation time decreased phosphorylation in the controls indicative of phosphatase activity. Two dimensional IEF and PAGE demonstrated that the phosphorylation of the 140K protein (pI = 6.1) was inhibited 89% by TAU while the 20K protein (pI = 5.6) was inhibited 71%. Guanidinoethanesulfonic acid, a TAU transport inhibitor, had a similar inhibitory effect on the phosphorylation of the 140K protein but no effect on the 20K protein. Thus the TAU effect was specific for the 20K protein. The inhibitory actions on phosphorylation suggest that TAU may have an effect on neuroactivity. (Supported in part by NIH grant EY04780).

505.3

IMMUNOCYTOCHEMISTRY OF THE TAURINE BIOSYNTHESIS ENZYME, CYSTEINE SULFINATE DECARBOXYLASE (CSD), IN THE CEREBELLUM. M. Tappaz*, K. Almaghini* and A. Remy* (SPON: D.L. Martin). Inserm U 171, Centre Hospitalier Sylon-Sud, 69310 Pierre-Benite, France.

An antiserum was produced against rat brain cysteine sulfinate decarboxylase (CSD), using as the immunogen an homogeneous fraction of liver CSD. This antiserum: a) quantitatively immunoprecipitated CSD activity, b) labelled one band (MW=51 kD) on immunoblots of a brain CSD-enriched fraction but none with a brain crude extract. In the cerebellum numerous immunolabelled cells were found in the white matter that were arranged like oligodendrocytes. Around the Purkinje cells many small satellite cells were immunostained that sent faintly labelled radial fibers through the molecular layer. Purkinje cells were not labelled at cell body or at the nerve endings. In the molecular layer no immunolabelled cells were found that could correspond to stellate cells. No immunopositive puncta typical of interneurone nerve endings were ever observed. All CSD-immunopositive cells were of glial nature. Biochemical determinations of CSD in glial cell-enriched fractions by a selective, sensitive immunotrapping assay revealed substantial CSD activity in astrocytes and oligodendrocytes. The glial localization of CSD in the cerebellum seriously challenges the view that taurine could be a neurotransmitter. As a new working hypothesis, we propose that taurine may be manufactured and released by glial cells and function as a general purpose regulator of nerve cells.

505.4

EVIDENCE FOR ^{3}H-ORG 2766 [A SYNTHETIC ACTH FRAGMENT] UPTAKE IN RAT HIPPOCAMPAL SYNAPTOSOMES. M.I. Davila-Garcia, N.M. Kheck, P.M. Whitaker-Azmitia, J.A.D.M. Tonnaer, and E.C. Azmitia. Washington Square Center for Neuroscience, New York University, Dept. Biology, New York, NY 10003.

The presence of specific receptor/binding sites for ACTH and its fragments have not yet been established. We have therefore, tested for the presence of uptake sites for ^{3}H-Org 2766 on hippocampal synaptosomal preparations. Tissues were obtained from young Sprague-Dawley female rats (250 gm), homogenized and centrifuged at 10,000 rpm. The pellet was resuspended in MEM with 5% glucose and ^{3}H-Org 2766 (35nM to 10uM) with or without excess unlabelled Org 2766 (to saturate specific uptake sites). The preparations were incubated for 20 minutes at 37^{o}C. The tissue was harvested on Whatman G/F filter paper. The filters were placed in 5 ml of scintillation fluid and counted. Our results showed a saturable low affinity uptake site for ^{3}H-Org 2766 into hippocamal synaptosomal preparations after a 20 minute incubation. Studies are under way to identify the ^{3}H-Org 2766 uptake into serotonergic neurons grown in culture. This research was supported by Organon International and NSF BNS 8812892.

505.5

ROLE OF ARACHIDONIC ACID (AA) METABOLITES IN THE RELEASE OF VASOACTIVE INTESTINAL PEPTIDE (VIP) IN MOUSE CEREBRAL CORTEX. J.L. Martin and P.J. Magistretti. Institut de Physiologie, Université de Lausanne, 1005 Lausanne - Switzerland.

In rodent cerebral cortex VIP is contained in a homogeneous population of radially-oriented bipolar interneurons. 4-aminopyridine (4-AP), a K^+-channel blocker, promotes a concentration- and Ca^{++}-dependent release of VIP from mouse cerebral cortical slices with a significant effect already at 50 µM. Over 70% of VIP release elicited by 4-AP (4APVR) is blocked by 2 µM tetrodotoxin (TTX). Mepacrine, an inhibitor of phospholipase A_2 (PLA_2) activity and hence of AA formation, inhibits 4APVR (IC50: 15 µM). Melittin (0.1-10 µg/ml) a PLA_2 activator promotes VIP release. Inhibition of AA metabolites of the lipoxygenase pathway by NDGA, ETYA and caffeic acid results in a concentration-dependent inhibition of 4APVR. Thus, the formation of AA metabolites of the lipoxygenase pathway appears to play a role in the release of a peptide in the mammalian CNS. Furthermore, these observations, taken together with the previously reported potentiation by prostaglandins of VIP-stimulated cAMP formation in mouse cerebral cortex (Schaad,N., Schorderet,M. and Magistretti, P.J. Nature, 328,637, 1987), indicate that AA metabolites may act at both the presynaptic (lipoxygenase metabolites) and postsynaptic (cyclooxygenase metabolites) levels to increase the "throughput" or "strength" of VIP-containing cortical circuits.

A Ca^{++}- dependent K^+- evoked VIP release (KVR) was also observed; KVR is not inhibited by nifedipine (10 µM), ω-conotoxin (1 µM) or Cd^{++} (100 µM), while Ni^{++} (1 µM) decreases KVR by over 60%. This pharmacological profile discards the involvement of L- and N-type Ca^{++} channels in KVR, in contrast to the release of other, non-peptidic, neurotransmitters which appears to be mediated by channels of the N-type.

505.6

THE EFFECT OF ADENOSINE ON TACHYKININ RELEASE FROM PERIFUSED ENTERIC NERVE VARICOSITIES. R.M. Broad*, T.J. McDonald* and M.A. Cook. Depts. of Pharmacology and Toxicology, and Medicine, Univ. of Western Ontario, London, Ont. N6A 5C1.

The release of tachykinins (TK's) from isolated enteric nerve varicosities may allow useful modelling of neural regulatory mechanisms. We have used perifused enteric nerve varicosities, prepared from the myenteric plexus of the guinea-pig ileum, to examine the release of TK's under flow conditions. Synaptosomes (P_2) were perifused with Locke's solution at 37°C and specific RIA's for Substance P (SP)- and Neurokinin A (NKA)-like immunoreactivity (LI) were performed on the effluent. Basal release of both SP-LI and NKA-LI was 10-40 fmol/mg protein/min. Depolarization-induced release, evoked by increment in $[K^+]_o$, was consistently 2-3 times basal levels. The adenosine receptor antagonist 8-cyclopentyl-1,3-dipropylxanthine (CPDPX, 1.0µM) augmented both basal and evoked release, suggesting that endogenous adenosine may tonically inhibit both release processes. Use of 5'-N-ethylcarboxamide-adenosine (NECA, 100nM) diminished only the evoked portion of TK release and this inhibition was blocked by CPDPX. Perifusion with zero Ca^{++} and 75µM EGTA essentially abolished K^+-evoked release. These experiments suggest that control of the release of endogenous TK's may involve adenosine receptor occupancy and demonstrate the viability of this approach in studying the release of neuroactive substances from nerve endings and the modulation of such release. Supported by M.R.C of Canada.

505.7

HYPERTONIC SOLUTIONS BLOCK PEPTIDE SECRETION AND AFTERDISCHARGES IN *APLYSIA* BAG CELL NEURONS. K.J. Loechner and L.K. Kaczmarek. Dept. of Pharmacology, Yale Univ. Sch. of Med., New Haven, CT 06510.

The bag cell neurons (BCNs) control egg-laying behavior in *Aplysia*. Upon stimulation, these cells generate a 20-40 min afterdischarge, during which several peptides are released, including egg-laying hormone (ELH). We have examined the effects of altering osmolarity on electrical activity and on KCl-evoked release of ELH, analyzed using HPLC. Depolarization of BCNs with isotonic high KCl (300mM) medium (partial replacement of NaCl with KCl, ~1200mosm) for 30 min evoked a pattern of release comparable to that observed during an discharge (n=3). In contrast, hypertonic high KCl (300mM) medium (~1750mosm) failed to induce release (n=4). Similarly, ASW made hypertonic with 600mM mannitol (~1840mosm) blocked KCl-evoked release of ELH (n=4). Increased osmolarity also affected BCN electrical activity. Hypertonic mannitol/ASW prevented normal discharges recorded in intact bag cell clusters. BCNs were either refractory to electrical stimulation or fired for less than 1 min (n=3). Moreover, discharges elicited in normal ASW were terminated within 1 min of exposure to hypertonic mannitol/ASW (n=4). The effects were reversible in that normal discharges were obtained 24 hr later in ASW. Addition of hypertonic mannitol/ASW (n=3) or hypertonic NaCl (300mM)/ASW (n=2) to single BCNs in primary culture attenuated action potentials. The inhibition was rapid and reversible with washout. In sum, hypertonic media inhibit both electrical activity and secretion in the BCNs, and we are investigating the degree to which these effects are interdependent.

505.8

RELEASE OF ANGIOTENSIN II IN RESPONSE TO A GRADED POTASSIUM ION STIMULATION, CHANNEL BLOCKERS AND CALMODULIN ANTAGONIST W-7. A.P. Gadbut, S.M. Cash*, W.P. Dagiantis*, J.A. Weyhenmeyer. Program in Neural and Behavioral Biology, College of Medicine, Univ. of Illinois, Urbana, IL 61801.

Although the central synthesis of angiotensin II (AII) has been well established, very little is known about the presynaptic release of this putative neurotransmitter. In this study, we utilized cultures of dissociated cells from fetal rat brain to examine the cellular and ionic properties of AII release. Graded concentrations of 0 to 59 mM K^+ in the presence or absence of 5 mM Ca^{++} were added to the cultured cells and the resulting AII release was measured by radioimmunoassay and HPLC. Levels of AII release increased from 1.4 ± .17 pg/mg protein to 17.25 ± 1.74 pg/mg protein with increasing concentrations of K^+. The cultures stimulated with a buffer containing 59 mM KCl/0 Ca^{++} released 3.94 ± 1.6 pg/mg protein AII, which was not significantly different from the baseline value of 4.9 ± 1.6 pg/mg protein. In a separate set of experiments, cultures were incubated with the calmodulin antagonist W-7, the sodium channel blocker TTX, the potassium channel blocker TEA, or calcium channel blocker verapamil prior to maximal stimulation with 59 mM K^+/5 mM Ca^{++}. Release was attenuated following preincubation with TTX, TEA, and W-7 to 0.66 ± 0.17 pg/mg protein, 5.73 ± 1.20 pg/mg protein and 0.47 ± 0.41 pg/mg protein, respectively. Preincubation with verapamil had no significant effect on AII release. The data indicate that potassium-induced AII release is a calcium-dependent process that requires the activation of calmodulin.

This study was supported by NSF grant BNS 17117.

PEPTIDES: BIOSYNTHESIS, METABOLISM AND BIOCHEMICAL CHARACTERIZATION IV

506.1

EXPRESSION OF NEUROPEPTIDE PROCESSING ENZYMES IN CULTURED NEURONS AND ASTROCYTES. R.S. Klein*, M.H.Vilijn, J.A.Kessler, L.D.Fricker. Dept. Neuroscience, Albert Einstein College of Medicine, Bronx, NY 10461.

The enzymes carboxypeptidase E (CPE) and peptidyl-glycine-α-amidating-monooxygenase (PAM) are involved in the processing of peptide hormones from their precursor molecules. In this study, we show that primary cultures of neurons and astrocytes secrete CPE enzymatic activity and express CPE and PAM mRNAs. Astrocytes were cultured from neonatal rat brains and neurons were cultured from e17 rat hypothalami. Astrocytes were treated with .1 mM 2'-deoxy-5-fluorouridine to prevent overproliferation and maintained in culture for three weeks prior to mRNA analysis. Cultured hypothalamic astrocytes have 4-5 fold higher levels of CPE and PAM mRNAs than cultured hypothalamic neurons. Both astrocytes and neurons express a single species of CPE mRNA (2.1 Kb) and two species of PAM mRNA (approximately 3.8 and 4.0 Kb). Astrocytes cultured from striatum, cortex, hippocampus and hypothalamus have high levels of CPE and PAM mRNAs and those cultured from cerebellum have low levels.

In situ hybridization studies performed on five day cultures of heterogenous populations of neurons and astrocytes from e17 hypothalami demonstrate the presence of CPE mRNA in many cells. Current studies are addressing the specific types of astrocytes expressing the mRNA encoding these neuropeptide processing enzymes.

506.2

EXPRESSION OF PEPTIDYLGLYCINE α-AMIDATING MONOOXYGENASE (PAM) IN THE CNS AND NEUROBLASTOMA X GLIOMA HYBRID NG108-15 CELL LINE. K. M. Braas, E. A. Thiele*, and V. May. Dept. Anatomy & Neurobiology, Univ. of Vermont College of Medicine, Burlington, VT 05405, Dept. of Neuroscience, Johns Hopkins Univ. School of Medicine, Baltimore, MD 21205

Peptidylglycine α-amidating monooxygenase (PAM) is a post-translational processing enzyme which catalyzes the formation of α-amidated bioactive peptides from their inactive glycine-extended precursors. Region specific expression of PAM is found in the CNS, with highest levels of soluble (142 ± 20 pmol/µg protein/h) and membrane-associated (138 ± 34 pmol/µg protein/h) PAM activity in the hypothalamus. Approximately 40-60% of total CNS tissue PAM activity was found in the particulate fraction. The neuroblastoma x glioma NG108-15 hybrid cell line expresses many neuronal characteristics, and has been used as a model neuronal system. NG108-15 cells expressed both soluble (141 ± 23 pmol/µg protein/h) and membrane-associated (181 ± 42 pmol/µg protein/h) activities. Approximately 30% of the total NG108-15 cell PAM activity was membrane-associated. Northern analysis demonstrated that CNS tissues and NG108-15 cells expressed PAM mRNA in the 4.2 and 3.8 kb size classes. Treatment of NG108-15 cells with 1 mM dibutyryl cAMP or 1 µM dexamethasone for 48 h resulted in increases in total PAM activity and PAM mRNA levels. Simultaneous treatment with both drugs produced increases in PAM activity and PAM mRNA levels greater than either drug treatment alone. Supported by DA 00266.

506.3

DIET AND URINARY EXCRETION OF CYCLO(HIS-PRO)-LIKE IMMUNOREACTIVITY (CHP-LI). C. Prasad and S. Eloby-Childress* (SPON: D.G. Kline). Lab. of Neurosciences, Pennington Biomedical Res Ctr, Baton Rouge and Dept. of Medicine, LSUMC, New Orleans, LA.

CHP is not only endogenous to many tissues and body fluids, but exogenous peptide is known to exhibit a number of biologic activities. Although CHP was initially discovered as a metabolite of thyrotropin-releasing hormone, many other sources--including dietary protein--have been suggested. We have examined, therefore, whether consumption of a diet rich in protein can lead to increased urinary excretion of CHP. To this end, six rats were kept on a equicaloric all carbohydrate-fat diet for 5 days and then switched to all protein (casein)-fat diet for another 5 days. Twenty-four hr urine samples were collected on day 4 and 5 of each diet, and samples were analyzed for CHP-LI and creatinine. Results show that there was a 68% increase in the urinary level of CHP-LI when animals were switched from carbohydrate to protein diet. In conclusion these data suggest that at least part of urinary CHP may be derived from dietary protein.

Diet	CHP-LI, ug/dl	Creatinine, mg/dl
Carbohydrate	5.8 ± 1.7	14.2 ± 0.5
Protein	9.9 ± 1.9	15.8 ± 0.9
p-value	0.004	0.06

506.4

THE SALMFamides: A NEW FAMILY OF NEUROPEPTIDES ISOLATED FROM AN ECHINODERM. M.R. Elphick*(1,2), D.A. Price (1) T.D.Les*(3) and M.C.Thorndyke*(2). 1. Whitney Lab, University of Florida, St. Augustine, FL 32086, U.S.A. 2. Biology Dept, R.H.B.N.C., University of London, Egham Surrey, TW20 OEX,U.K. 3. Beckman research Institute, Duarte, CA 91010, U.S.A.

Three novel neuropeptides, SALMFamide, GFNSALMFamide and SGPYSFNSGLTFamide, have been isolated from radial nerve extracts of the starfish Asterias. The peptides were purified by HPLC and radioimmunoassay using an antibody to pQDPFLRFamide (a member of the molluscan FMRFamide neuropeptide family) and identified by sequencing and molecular weight determination (FABMS). SGPYSFNSGLTFamide was present in both A. rubens and A. forbseii whereas SALMFamide and GFNSALMFamide have so far only been identified in A.rubens and A. forbseii respectively. We propose that these three peptides are members of a family of structurally related neuropeptides, the "SALMFamides".

An earlier immunocytochemical study using FMRFamide antibodies (Elphick et al, 1989, Biol. Bull., in press) revealed immunoreactivity throughout the starfish nervous system that was presumably due to the presence of SALMFamides. We are currently testing synthetic SALMFamides on a number of echinoderm neuromuscular preparations.

506.5

FLUORESCENCE MICROSCOPY FOR THE MEASUREMENT OF AMINO ACIDS AND NEUROPEPTIDES BY CAPILLARY ZONE ELECTROPHORESIS. L. Hernandez*[1], N. Joshi*[2] & J. Martin*[2] (SPON: Z. Horovitz). [1]Laboratory of Behavioral Physiology, Medical School, [2]Department of Physics, University of Los Andes, Venezuela

Fluorescence microscopy was used to improve the sensitivity detection level of primary amine compounds separated by capillary zone electrophoresis. A capillary carrier was built and set on the platina of a fluorescence microscope. A fused silica tube 1 meter long and 20 um inside diameter was filled with 0.05M sodium tetraborate buffer at pH 8.3 and inserted into the capillary carrier. An uncoated section of the capillary was centered and focused. A mixture of 5 amino acids (serine, valine, leucine, isoleucine and treomine) or 5 neuropeptides (neurotensin, metenkephalin, angiotensin and sulfated and unsulfated cholecystokinin-8) were derivatized with fluorescamine and electrokinetically loaded (10 KV during 15 seconds) into the capillary. The sample was run at 30 KV during 30 minutes. The detection level of this system (3:1 signal to noise ratio) was 3.2 pg in column. Sensitivity was considerably improved by the addition of a chopper and a locking amplifier. With the frequency set at 200 Hz the noise was reduced one order of magnitude without affecting the magnitude of the signal. We conclude that the combination of epillumination and a locking amplifier lowers the detection level of the fluorescence detection method for capillary zone electrophoresis.

506.6

ISOLATION AND IDENTIFICATION OF RAT BRAIN RAS P21'S AS POTENTIAL CYSTEINE PROTEINASE INHIBITORS

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Brain is a rich source of ras P21 protooncogenes having G-protein-like properties which are thought to be involved in neuronal growth and differentiation. Recombinant P21's act as potent cysteine proteinase inhibitors (CPI's) attributable to presence of repeated cystatin consensus-like sequences. To determine if ras-CPI's co-purify with other endogenous inhibitors, rat brain and spinal cord were extracted at pH 10.6, heat treated, and purified as described by Marks et al. (Arch. Biochem. Biophys. 267:448-458, 1988). CPI fractions eluted from alkylated papain Reacti-gel with 2 M sodium thiocyanate incorporated 29.25 pmol GTPγ-[^{35}S]/mg protein representing an enrichment of 127 fold. SDS-PAGE separation and transfer to PVDF membrane revealed presence of 21-24 kDa bands binding GTPγ-[^{35}S]. Ras-like proteins were differentiated from low M_r cystatins and high M_r kininogens by immunoblotting using specific antisera such as MAb Y13-259. Results will be compared to ras G-proteins purified by conventional chromatographic procedures. Availability of affinity-purified ras P21-CPI's will facilitate studies on their roles in CNS protein turnover.

Supported in part by Grant NIDA-4178.

506.7

NEUROTENSIN COMPLEXES WITH DOPAMINE AND N-PROPYLAPOMORPHINE. D.K. Adachi*, P.W. Kalivas and J.O. Schenk (SPON: R. Quock). Dept. of VCAPP, Washington State University, Pullman, WA 99164-6520.

We have previously demonstrated that neurotensin (NT) complexes with dopamine (DA) with a stoichiometry of 1:1 and K_D = 3.9 x 10^{-8}. NT is a peptide with unique properties in that it appears to activate DA neurons at the level of the cell bodies but blocks the behavioral effects of DA release in the accumbens, a DA axonal terminal field. Although NT possesses some neuroleptic-like properties, it does not displace the in vitro binding of the DA antagonist spiperone, yet has been shown to decrease the affinity of the DA agonist, N-propylapomorphine (NPA) for striatal binding sites. We now report that complexation between NT and NPA occurs with a stoichiometry of 1:1 (K_D = 1.3 x 10^{-8}). Utilizing molecular modeling software designed to predict the conformations of peptides in aqueous solution, we have found that the structure of NT can form a highly basic pocket containing -Pro^7-Arg^8-Arg^9-Pro^{10}-. The Arg-Arg moiety may form and acid-base complex with the catechol moiety of DA and other catechols. An intact catechol moiety appears to be critical for the complexation reaction as 3-methoxytyramine, which possesses only a single free hydroxyl group, was observed to possess a much weaker interaction (K_D = 1.3 x 10^{-5}). The fact that NPA possesses an intact catechol moiety may provide a mechanism through which complexation with NT can occur and decrease NPA binding.

506.8

BOVINE PITUITARY MULTICATALYTIC PROTEINASE COMPLEX CONTAINS A TIGHTLY ASSOCIATED cAMP-DEPENDENT PROTEIN KINASE. M.E. Pereira* and S. Wilk. Dept. of Pharmacology , Mount Sinai School of Med. of CUNY, New York, N.Y.10029.

Multicatalytic proteinase complex (MPC), a high molecular weight (700 kDa) proteinase contains three distinct catalytic activities (Wilk and Orlowski, J. Neurochem. 40:842, 1983). It constitutes a major non-lysosomal proteolytic system in eukaryotic cells, and may function in processing of biologically active peptides. We report that a highly purified bovine pituitary MPC, giving a single protein band in PAGE under non-dissociating conditions, contains a tightly associated cAMP-dependent protein kinase (PK-A). The kinase phosphorylated a synthetic substrate (Kemptide) in the presence of 10 µM cAMP (10-fold stimulation) SDS/PAGE of purified MPC subjected to phosphorylating conditions in the presence of [g amma ^{32}P] ATP revealed phosphorylation of at least three components of the MPC as well as two bands with molecular masses of 45 and 55 kDa. The 55 kDa band was photoaffinity labelled with [^{32}P] 8-N_3-cAMP and likely represents the regulatory subunit of PK-A. The kinase is not an integral part of the complex and could be partially separated from the complex by gel filtration and gel electrophoresis. Interaction between MPC and the kinase is indicated by the observation that kinase activity was stimulated 3-fold by Cbz-Gly-Gly-Leu-pNA, an MPC substrate. Activation of the MPC-associated kinase by a proteinase substrate and the phosphorylation of MPC by the associated kinase suggests functional interaction of the two systems. (Supported by a Training Grant 5T3 DA-07135 to M.E.P.; NS-17392, and Res.Sci. Award MH-00350 to S.W.)

506.9

INTERACTIONS OF ACETYLCHOLINESTERASE (AChE) WITH PROTEASE INHIBITORS. M.R. Emmerling* (SPON. J. Chan). Dept. of Neurology, Univ. of Wisconsin, Madison, WI 53706

The alleged proteolytic nature of AChE (*see Chubb, 1984, in Cholinesterases - Fundamental and Applied Aspects, pp. 345-359*) was investigated by testing the affinity of the enzyme for the protease inhibitors benzamidine, pepstatin A, α_2-macroglobulin, soybean trypsin inhibitor, and lima bean trypsin inhibitor coupled to beaded Sepharose. Bovine serum AChE binds to all the inhibitors tested except α_2-macroglobulin. Three to ten times more AChE activity binds to columns of benzamidine and pepstatin A than to columns of soybean and lima bean trypsin inhibitors. Studies using just benzamidine and pepstatin A beads show that binding is not a universal property of all AChE; isoforms of AChE from mouse serum, brain and skeletal muscle bind to the inhibitors to varying degrees or not at all. AChE binding to the protease inhibitor beads is reduced by benzamidine, while the binding in the presence of pepstatin A and other protease inhibitors is the same as control levels or is increased. Inhibitors of AChE activity (edrophonium, BW284C51, atropine, curare and gallamine) reduce the amount of enzyme binding to benzamidine and pepstatin A beads. The results suggest that some AChE may possess sites to which inhibitors of serine and acid proteases bind, and that this interaction is influenced by inhibitors of AChE activity. These results are consistent with the notion that AChE may interact with and be involved in the breakdown of neuropeptides. (Supported by NSF Grant BNS-8719716 and NIH-NINCDS Grant NS26513-01)

506.10

VOLTAGE-DEPENDENT Ca^{2+} CHANNELS MODULATE GALANIN INHIBITION OF THE MUSCARINIC STIMULATION OD PHOSPHOINOSITIDE TURNOVER IN RAT VENTRAL HIPPOCAMPUS. S. Consolo+*, G. Fisone+°, T. Bartfai+° and E. Palazzi+* (SPON: F. Clementi). *Mario Negri Institute, Milan, Italy, and °Arrhenius Lab, S-106 91, Stockholm, Sweden.

Galanin (GAL) inhibits the breakdown of phosphoinositides (PI) stimulated by carbachol in miniprisms from rat ventral hippocampus. Ca^{2+} influx promoted by K^+ depolarization or Bay K 8644, a selective agonist of the L-type voltage-dependent calcium channel (VSCC) prevented the inhibitory effect of GAL. Blockade of the L-type channel with nifedipine (1μM) potentiated the inhibitory effect of GAL without affecting muscarinic stimulation of PI breakdown. Blockade of all VSCC with 200μM Cd^{2+} reduced muscarinic receptor mediated PI breakdown by 50% and prevented the inhibitory effect of GAL (1μM). Cd^{2+} ions at 20μM concentration still abolished the GAL's effect. ω-conotoxin, 2μM, which blocks the L- and N-types of VSCC, by itself inhibited carbachol mediated PI breakdown by about 25% and when added before GAL it prevented the inhibitory effect of the peptide. The properties of the VSCC involved in the inhibitory action of GAL coincide with those described for the N-type VSCC (Research Council Grant 87.00031.44, Rome, Italy).

506.11

BOVINE PINEAL THREONINE-RICH DECAPEPTIDE INCREASES ANTERIOR PITUITARY DOPAMINE IN MALE MICE. B. Benson. Department of Anatomy, University of Arizona, Tucson, AZ 85724.

During the course of large scale purification of bovine pineal peptides with mouse mammary milk-ejection activity (ME-activity), a peptide was identified which increased anterior pituitary dopamine (DA) in mice. Purification from more than five kg of defatted pineals was accomplished by homogenization in 0.2 N acetic acid, centrifugation (10,000 X g/20 min) at 4° C, Amicon ultrafiltration and Sephadex G-25 gel filtration. Subsequent isolation was achieved by serial semipreparative HPLC on C-8 columns with ternary gradient mobile phases consisting of acetonitrile: methanol and ammonium acetate in trifluoroacetic acid. The primary structures of oxytocin (OT) and vasopressin were confirmed by amino acid and microsequence analyses. A 210 nm absorbance peak was revealed with chromatographic properties closely related to those for OT, but without ME-activity. When injected into CD-1 male mice, ~1.0 μg of the peptide significantly increased anterior pituitary DA at 15 and 30 min postinjection. Preliminary amino acid analysis revealed a Thr-rich decapeptide. Supported by N.I.H. grant #HD 19521.

506.12

ISOLATION AND CHARACTERIZATION OF A NOVEL OVINE HYPOTHALAMIC POLYPEPTIDE WHICH STIMULATES ADENYLATE CYCLASE IN RAT PITUITARY CELL CULTURES. A. Miyata*, G. Katsuura*, P.E. Gottschall*, R. Dahl*, D.H. Coy*, M. Fujino* and A. Arimura, U.S.-Japan Biomed. Res. Labs., Tulane Univ. Hebert Cr., Belle Chasse, LA 70037; Depts. of Medicine & Anatomy, Tulane Univ. Sch. of Med., New Orleans, LA 70112; Takeda Chemical Industries, Tsukuba Labs., Tsukuba, Japan.

A novel neuropeptide with 38 residues which stimulates adenylate cyclase (AC) activity in the rat anterior pituitary cell cultures was isolated from ovine hypothalamic tissues and named as PACAP38. The N-terminal sequence [1-23] of this peptide shows homology with peptides of the secretin/glucagon family, especially with VIP (78%). Synthetic PACP38 is, however, 100 times more potent than VIP and comparable to CRH in terms of AC activation in rat pituitary cell cultures. Another AC activating substance distinct from PACAP38 or other known hypophysiotropic hormones was also found and effort was made to purify this peptide using AC activation in rat pituitary cell cultures as an activity parameter during purification. The starting material was the side fraction in the CM cation exchange chromatography of ovine hypothalamic extract (2400 g) obtained during purification of PACAP38. After two steps of a reverse phase HPLC, the peptide was isolated in a pure form. The amino acid sequence of this peptide was revealed to be a [1-27]-NH_2 of PACAP38. PACAP38 contains a signal sequence for proteolytic processing and amidation [-Gly^{28}-Lys^{29}-Arg^{30}-], suggesting the presence of PACAP27-NH_2. However, the final yield of PACAP27-NH_2 was only 1/10th that of PACAP38. PACAP27-NH_2 was also synthesized and found to exhibit a similar AC activating potency as PACAP38. It is noteworthy that there are two forms of PACAP are present in the hypothalamus. A broad range of physiological and morphological experiments are now in progress to elucidate the physiological roles of PACAP38 and PACAP27-NH_2. (Supported in part by NIH grants DK09094 and DK30167.)

INGESTIVE BEHAVIORS VI

507.1

AMILORIDE-SENSITIVE SODIUM CHANNELS AND SALT PREFERENCE OF PREWEANING RATS. S.I. Sollars* & I.L. Bernstein. Dept of Psych, Univ of Washington, Seattle, WA 98195.

The sodium chloride (NaCl) preference of preweaning rats differs significantly from that displayed by adults, particularly with regard to their preference for hypertonic NaCl solutions. Recent evidence indicates that the behavioral and electrophysiological response of the mammalian gustatory system to NaCl is dependent upon a sodium transport system which is specifically blocked by lingual application of the sodium transport blocker amiloride. Postnatal maturation of amiloride-sensitive sodium transport mechanisms in the taste bud could contribute to age-related changes in NaCl preference of rats (Hill & Bour, 1985). The present study investigated the effects of lingual application of amiloride on NaCl intake of preweaning rats. Preexposure solutions (amiloride hydrochloride or water) and test solutions (1%, 2%, 3% NaCl, distilled water and 2% ammonium chloride (NH_4Cl) were delivered through intraoral cannulae inserted under the animals' tongues. Weight gain during test infusions was used as an index of ingestion and expressed as a percent of body weight. At 10-days of age rats displayed a strong preference for 2% and 3% NaCl solutions over water which was significantly suppressed by amiloride preexposure. Amiloride preexposure was without effect on intake of distilled water and a strong preference for 2% NH_4Cl was unaffected by amiloride. These results point to the existence of functional amiloride-sensitive sodium channels in the gustatory system of the 10-day-old rat. Additional studies evaluated developmental changes in the sensitivity to amiloride between birth and weaning.

507.2

NEUROTENSIN STIMULATES WATER INTAKE. Karen R. Hendricks* and John B. Simpson, Department of Psychology, University of Washington, Seattle, WA 98195.

A preliminary report (Evered, M.D., Proceedings Canadian Federation of Biological Societies Abstracts, 21, 1978) indicates that neurotensin (NT) causes an increase in water intake. We have replicated this experiment and extended this finding. We show that the action of NT does not occur independently of other neural pathways.

Long-Evans rats with lateral ventricular cannulas rapidly drank increasing amounts of water over the range of 72.9 fmol (1 ml) to 72.9 pmol (4.8 ml) of NT over a 30 minute period, with all latencies less than 3 minutes. Peripheral administration of 72.9 pmol of NT had no effect.

In order to test whether NT acted in concert with other neural pathways, animals were pretreated with receptor blockers in dosages that were 100 times the molal concentration of NT (59.78 pmol). NT drinking was 69% reduced by atropine and 60% reduced by fluphenazine pretreatment. NT drinking was 36% enhanced by naloxone pretreatment and not affected by Sarthran (an angiotensin II antagonist) pretreatment.

Thus, NT is a potent CNS dipsogen and depends in part on dopaminergic, cholinergic and opioid mediation.

507.3

RATE OF SODIUM LOSS PREDICTS SALT APPETITE. S.P. Frankmann, C. Assanasen*, J. Reilly* and T. Siff*. Bourne Lab, Dept. Psychiatry, NY Hosp-Cornell Med Ctr, White Plains, NY 10605 & Monell Ctr., Phila, PA 19104.

Following a first sodium (Na+) depletion by a large dose of Lasix (10 mg) a second depletion produces a more rapid and a larger 120-min intake of Na+ with no increase of Na+ loss. To determine if multiple small Na+ depletions would produce similar effects, 12 female hooded rats received 3 vehicle (V) or Lasix (L, 1 mg) treatments once every 7 days. Urine volume and Na+ concentration were measured at 2 and 24h after injection. At 24h, intakes of 0.3M NaCl were recorded over 120-min and were increased in L compared to V for all depletions. Compared to the first depletion, there were significant increases of 0.3M NaCl intake at 5,10 and 15 min for the L group (Table, *p<.05).

	5'	10'	15'	30'	45'	60'	120'
First	1.4	1.8	2.3	3.0	3.0	3.0	3.5
Second	2.5*	2.8*	2.8	3.1	3.3	3.3	4.0
Third	2.6*	2.9*	3.0*	3.0	3.0	3.2	3.7

Thus, Na+ intake was more rapid but total intake was not increased. 24h Na+ loss did not change, but 2h losses were reduced after the second and third (.60, .54, mEq) compared to the first (.82) treatment. Thus, rate of sodium loss was slower after the first depletion. We suggest that the slower rate of Na+ loss after repeated administration of 1 mgL prevents the increase in 120-min intake of Na+ observed after 10 mgL, but not the more rapid intake of Na+.

507.4

SODIUM APPETITE IS NOT ENHANCED DURING LACTATION IN RATS Edward M. Stricker, Joseph G. Verbalis, and Edda Thiels. Departments of Behavioral Neuroscience and Medicine, and Center for Neuroscience, University of Pittsburgh, Pittsburgh, PA 15260.

Fifty years ago, Richter and Barelare reported a small but statistically reliable increase in the intake of 3% NaCl solution by lactating rats maintained on "cafeteria" diet (Endocrin., 23:15, 1938). However, in recent experiments we found no difference in the intake of 0.5 M NaCl solution between virgin and lactating female rats when they were maintained either on sodium-rich (NaR) or sodium-deficient (NaD) diets. Sodium losses in urine virtually disappeared in lactating NaD rats, whereas sodium concentration in milk was unchanged and their pups (8/litter) grew normally. To determine whether lactating rats are able to develop sodium appetite, they were injected sc with 30% polyethylene glycol (PEG) solution (5 ml), to reduce plasma volume isosmotically, 8 days postpartum. The PEG treatment rapidly enhanced NaCl intake to levels above those of PEG-treated virgin NaD rats, and both NaD groups consumed more NaCl than did lactating and virgin NaR rats. These observations indicate that enhanced NaCl intake is not an integral feature of lactation despite the substantial sodium loss during milk transfer to the young, the marked increase in renal sodium conservation, and the evident ability of lactating rats to experience sodium appetite appropriately in response to a pronounced, acute need. (Supported by NIMH research grant MH-25140.)

507.5

PREOPTIC AREA AND ANGIOTENSIN-INDUCED SALT APPETITE. D.A. Fitts and D.B. Masson*. Dept. of Psychology, Univ. of Wash., Seattle, WA 98195.

Salt appetite induced by angiotensin II (ANG) in rats is mediated by forebrain areas other than the subfornical organ (SFO) (Fitts & Masson, Behav. Neurosci., in press). Many experimenters have infused ANG or its blockers into the preoptic recess of the third ventricle (IIIV) to manipulate salt appetite, but none has identified a preoptic angiotensinoceptive area for salt appetite using tissue-infusion or lesion techniques. In the present experiments, ANG infusions into tissue immediately rostral to the IIIV and within 0.4 mm of the organum vasculosum laminae terminalis (OVLT) increased intake of both 0.3 M NaCl and water. Electrolytic lesions targeted for the same area (n = 30) reduced ANG-induced salt appetite during 40 mg/day oral captopril (CAP) from 13 ml/day in sham-lesioned rats (n = 18) to 9 ml/day (p < .05). The lesions did not affect enhanced water intake to CAP, sodium excretion after the lesion, or plasma protein, Na, K, osmolality, or hematocrit. The lesions most often damaged the OVLT, the ventral median preoptic nucleus (MnPO), and/or the medial preoptic area. Seven rats with complete lesions of the OVLT did not reduce saline intake to CAP. In fact, of 19 rats having substantial damage to the MnPO, intake to CAP was reduced the most when the lesion spared the OVLT: rats drinking less than the MnPO subgroup median, 8.2 ml, averaged 26% destruction of the OVLT compared with 77% in rats drinking more than the median intake (p < .01). Similar infusions (these experiments) or lesions (Thunhorst, et al, AJP, 252, R404, 1987) of the SFO affected water intake but not salt appetite. We conclude that ANG receptors in tissues surrounding the most anteroventral portion of the preoptic recess of the IIIV are important for salt appetite to ANG, but we cannot confirm a role for the OVLT in the enhanced salt appetite to CAP. Supported by NS-22274 to D.A. Fitts.

507.6

WATER INTAKE FOLLOWING VARIOUS CHALLENGES IN A DESERT RODENT, THE SPINY MOUSE (A. CAHIRINUS). D.A. Czech and R. Prince*. Dept. Psychol. Marquette Univ., Milwaukee, WI 53233.

Male mice were exposed to several drinking challenges in within-subjects designs. Conditions were: Hypertonic NaCl (10 ml/kg of 1 M NaCl) and 0.15 M NaCl control ip; 24-hr water deprivation and non-deprived control; d,l-isoproterenol HCl (50, 100, 400 & 800 µg/kg) and 0.15 M NaCl vehicle sc; Angiotensin II (AII) (200, 600 & 1200 µg/kg of ^{5}Ileu-AII) and vehicle sc; Polyethylene glycol (PEG) (30 ml/kg of 25%, w/v, of PEG; M.W.≈20K) and vehicle sc.

Deionized water intake was monitored electronically at 1-5 min intervals in the home cage over a 2-6 hr test period (no food present) during the light part of the L/D cycle. Drinking tests were separated by 3-4 days. Cumulative intakes were evaluated with repeated measures ANOVAs and Dunnett's procedures; alpha level was set at $p < 0.05$.

As expected, water deprivation and hypertonic saline led to robust drinking. PEG was also an effective stimulus. A modest, but significant, intake difference was evident within the first hr; 7 of 13 mice drank within 60 min following PEG. All drank within 3 hr of PEG injection, and mean cumulative intake reached 2.0 ml (compared to 0.14 ml for vehicle) by the end of 6 hr. Isoproterenol stimulated significant intake at doses from 100 µg/kg; while latencies were somewhat variable, nearly all drinking occurred within the first hr. A relatively high dose of AII (600 µg/kg) was needed to reach marginal significance at hr 2. Both latency to drink and time course were markedly variable.

507.7

ANGIOTENSIN II PARTIALLY REVERSES CAPTOPRIL-INDUCED INHIBITION OF FOOD-RELATED DRINKING IN THE RAT. E.S. Kraly and E. Cornelson*. Dept. of Psychology, Colgate Univ., Hamilton, NY 13346.

The ability of angiotensin II (ANG II) to reverse the inhibitory effects of captopril (CA) on food-related drinking was examined in adult male Sprague-Dawley rats (n=11) ingesting pelleted chow and water after 24-hr food deprivation. Captopril (100 mg/kg s.c. at 15 min prior to eating), in a dose sufficient to abolish synthesis of ANG II in peripheral circulation and brain, decreased ($p<0.05$) 75-min water:food ratio (W:F) from 1.7 to 1.1 ml/g. A dose of s.c. ANG II (5 mcg/kg), subthreshold ($p>0.10$) for eliciting drinking when given to nondeprived rats that were not eating, restored ($p<0.05$) in CA-treated rats 75-min W:F to 1.7 ml/g when ANG II was given 1 min prior to eating. Ingestive behavior after ANG II plus CA was not the same as baseline conditions (i.e., rats treated with 0.9% NaCl), however, because ANG II decreased ($p<0.01$) 60-min food intake. In contrast, the same dose of ANG II given together with CA 15 min prior to eating increased ($p<0.02$) 15-min preprandial water intake without significant ($ps>0.05$) effect on W:F or food intake (compared to CA alone). Thus, a dipsogenically-subthreshold dose of s.c. ANG II (a) only partially reverses inhibition of food-related drinking caused by CA-induced total blockade of synthesis of ANG II, and (b) appears able to elicit drinking when hungry rats are ready to begin eating.

507.8

ANATOMICAL CORROBORATION OF CELLULAR, BUT NOT FIBER DAMAGE AFTER IBOTENIC ACID LESIONS OF THE LATERAL PARABRACHIAL NUCLEUS THAT PRODUCE OVERDRINKING. G.L. Edwards, T.G. Beltz* and A.K. Johnson. Depts. of Psychol. and Pharmacol. and the Cardiovascular Ctr., Univ. of Iowa, Iowa City, IA 52242.

We have reported that rats with ibotenic acid (IBO) lesions of the lateral parabrachial nucleus (lPBN) ingest increased amounts of water after peripheral administration of angiotensin II or isoproterenol (Fed. Proc. 46, 1987). These data suggest that neurons of the lPBN are involved in an ascending hindbrain pathway that acts to inhibit drinking to extracellular thirst challenges. In these studies we have utilized cholera toxin-conjugated HRP (CT-HRP) as an anterograde tracer to label fibers that pass through the lPBN in an attempt to verify that fibers of passage remain intact after IBO lesions.

We injected CT-HRP into the area postrema and adjacent nucleus of the solitary tract (NTS), a region reported to send axons to the lPBN and midbrain (J. Comp. Neurol. 234: 344, 1985), in rats with IBO lesions of the lPBN and unlesioned control rats. When we compared the distribution of HRP labelled fibers in lesioned and control rats we found that HRP labelled fibers appear to pass through the lesioned lPBN and continue to more medial and rostral structures. These data provide further evidence that neuronal loss in the lPBN, and not damage to fibers of passage, is critical to the enhanced water intake after extracellular thirst challenges observed in rats with lesions of the lPBN. (Supported by NIH HL14388)

507.9

ROLE OF CENTRAL α_1 AND α_2-ADRENOCEPTORS ON THE DIPSOGENIC AND CARDIOVASCULAR EFFECT OF ANGIOTENSIN II. E.Colombari*, W.A. Saad, L.A.A. Camargo*, A. Renzi*, L.A. De Luca Jr.*, J.V. Menani* and W. Abrao Saad*.Dep. of Physiology, School of Dentistry, UNESP, Araraquara, SP, Brazil, and Dep. of Surgery, School of Medicine, USP, São Paulo, SP, Brazil.

Intracerebroventricular (ICV) injection of angiotensin II (AII) in rats increases the blood pressure (BP), heart rate (HR) and water intake (WI). The catecholamines of the central nervous system (CNS) are involved in these effects. We studied the effects of the α_1 and α_2-adrenoceptors on the pressor, tachycardic and dipsogenic action of AII injected ICV in rats. ICV injection of AII (12 ng) produced an increase in BP, HR and WI. Previously ICV treatment with α_2-adrenoceptor agonist, clonidine (20, 40, 80 and 120 nmol) decreased the pressor,tachycardic and dipsogenic effects of AII. The α_1-adrenoceptor antagonist, prazosin (80 and 120 nmol) injected previously also reduced the pressor and tachycardic effect of AII, but not the dipsogenic response. The data confirm the role of central adrenergic system in the mediation of thirst and cardiovascular responses induced by AII. They also suggest that the central α_1-adrenoceptors is involved only in cardiovascular responses produced by central AII, whereas all actions of AII can be modulated by the α_2-adrenoceptors.

Research supported by FAPESP (88/9188-6).

507.10

THE EFFECT OF CHOLECYSTOKININ-OCTAPEPTIDE (CCK) AND DISTENSION ON FOOD INTAKE AND BEHAVIOR IN RATS. S. Wager-Srdar and A.S. Levine. Department of Medical Physiology, University of Calgary, Calgary, Alberta T2N 4N1 and V.A. Medical Center, Minneapolis, MN 55417.

CCK's satiating effect may be due to gastric distension, secondary to inhibition of gastric emptying. To evaluate the interaction between distension and CCK on food intake and behavior we observed sleeping, resting, exploring, moving, grooming, chewing and eating behavior in 24 hr food deprived rats following saline or CCK (5µg/kg) ip and intubation of a 1.5% guar test meal (0, 1.5, 3 & 6 ml). The rats were observed in their home cages with chow, no chow or a novel object present. CCK increased sleeping (132%) and moving (31%) and decreased grooming (28%) and eating (33%) compared to the saline group when chow was present, $p < 0.05$. In the presence of a novel object, CCK decreased exploring (88%) and chewing (80%) and increased sleeping (198%), $p < 0.05$. CCK and distension did not interact to affect any behavior in the presence of chow or a novel object. Without chow present, CCK + test meal increased resting 139% compared to the saline group and there was an interaction between CCK and gastric volume on this behavior, $p < 0.05$. CCK had a sedating effect in rats during all conditions; however, exploration and movement may be affected differently by CCK in rats in the presence or absence of food.

507.11

EFFECT OF LATERAL CEREBRAL VENTRICULAR (LV) INJECTION OF CCK-8 ON SHAM (SF) AND REAL FEEDING (RF) IN RATS. M.A. Della-Fera and B.D. Coleman.* Washington U. Sch. Med., St. Louis, MO 63110

CCK-8 administered IP suppresses both RF and SF of liquid diet in fasted rats (Gibbs et al, 1973). Although centrally administered CCK has been shown to suppress feeding under a variety of paradigms, its effect on SF has not been studied. Our objective was to determine whether CCK-8 administered into the LV suppressed both RF and SF. We first showed that as little as 43 pmol (50 ng) CCK-8 injected into the LV suppressed chow intake for up to 1 hr in 6 hr fasted rats (60 min FI for control, 43 pmol, 86 pmol and 430 pmol, respectively: 2.6+.9, .8+.3, 1.0+.3, .6+.3 g; all CCK treatments less than control $p<.05$) . SF and RF tests were carried out in rats prepared with gastric cannulas and adapted to drinking liquid diet with the cannulas open (SF) or closed (RF). IP injection of 4 ug/kg CCK-8 suppressed RF by 67% and SF by 80% after 30 min ($p<.05$). LV bolus injection of 43 and 430 pmol CCK-8 caused dose-related decreases in RF: after 30 min, intakes were suppressed 20 and 54% ($p<.05$), respectively (control intake: 12 + 2 ml). LV injection of CCK-8 had no effect on SF: at 30 min intakes were 22 + 3, 22 + 4 and 27 + 7 ml with 0, 43 and 430 pmol, respectively. These results suggest that CCK of peripheral origin may activate feeding-related pathways not accessed by CCK administered into the LV. Supported by NIH NS20000.

507.12

CCK concentration <CCK> changes in specific brain areas after intestinal infusion of liquid diet in rats. J. Koch, M.A. DellaFera, H. Marshall, & C.A. Baile.(SPON: C.L. McLaughlin) Washington U. Sch. Med, St. Louis, MO 63110

Changes in <CCK> in specific brain sites occur with feeding and after peripheral (IP) injection of CCK-8 in rats, and there are important circadian influences, as well. The objective of this experiment was to determine the changes in brain <CCK> occurring as a result of intraduodenal infusion of liquid diet (Ensure) in rats infused during the dark (D) or light (L) phase of the circadian cycle. Male Sprague Dawley rats (N = 32) were implanted with intraduodenal catheters extending 4 cm beyond the pylorus. Half (16) were adapted to infusion procedures during L and the other half, during D. Rats in each group were randomly assigned to saline (S) or Ensure (E) infusion (1.0 ml infused over 2 min). They were sacrificed 5 min after infusion. Changes in <CCK> occurred in 3 areas: In the supraoptic n., <CCK> was higher ($p<.01$) in D compared to L rats, irregardless of infusion treatment. In the dorsal parabrachial n. <CCK> was higher ($p<.05$) in E compared to S infused rats, irregardless of L/D phase. In the dorsal motor vagal n., <CCK> was higher in LE compared to LS rats ($p<.05$); there was no infusion effect in D rats. These findings indicate that nutrient stimulation of the intestine elicits a different pattern of changes in brain <CCK> than either meal ingestion or IP injection of CCK. Supported by NIH NS20000.

507.13

CCK RECEPTOR BLOCKER CR1409 INCREASES FEEDING AND REVERSES CCK-8 MEDIATED SATIETY AFTER LATERAL CEREBRAL VENTRICULAR (LV) INJECTION IN SHEEP. C.A. Baile, M.A. Della-Fera, D.R. Brown & B.D. Coleman. Washington U Sch Med, St. Louis, MO 63110

There is considerable evidence that CCK peptides in brain have a physiological role in satiety. In fasted sheep, doses within a physiological range were shown to decrease feeding during LV injection, and CCK antibody infused into the LV prolonged feeding and increased food intake in satiated sheep. Our objective in these studies was to investigate the role of brain CCK receptors in satiety occurring after feeding, and as mediated by exogenous CCK-8. In the first experiment, LV injection of 0 or 500 pmol CR1409 was given at the end of a 15 min meal. Subsequent feed intakes increased after CR1409 and were significantly greater than control by 2 hr. In the second experiment, 2 hr fasted sheep were given 0 or 20 pmol CR1409 into the LV, and continuous LV injection of 0 (sCSF) or .64 pmol/min CCK-8 was begun 15 min before feed was returned. CCK-8 alone decreased feed intakes throughout the 3 hr period ($p<.05$); but with the combination of CR1409 and CCK-8, the suppression of feeding was reversed. In the fasted sheep CR1409 alone did not significantly increase feeding. These findings indicate that by blocking brain CCK receptors, the satiety effects of CCK in the brain can be reversed. CR1409 was supplied by Rotta Research Laboratorium. Research supported by NIH NS20000.

507.14

EFFECTS OF SULFATED AND NON-SULFATED CHOLECYSTOKININ ON PYLORIC CONTRACTILITY IN THE DEVELOPING RAT. G.J. Schwartz, T.H. Moran and P.R. McHugh, Dept. of Psychiatry, Johns Hopkins Univ. Sch. of Med., Baltimore, MD 21205.

Cholecystokinin inhibits food intake and gastric emptying in adult and developing rats as soon as postnatal day 1, while non-sulfated CCK (D-CCK) does not. In the developing rat, CCK binding occurs throughout the upper GI tract, becoming more restricted with age until it resembles the adult pattern, localized to the circular muscle of the pylorus. These studies suggest that CCK binding to pyloric sites may contribute to CCK's ability to suppress food intake and gastric emptying. To determine CCK's action on the developing rat pylorus, in vitro contraction of pyloric segments of rats aged 1, 3, 6, 10, 15, and 20 postnatal days was measured in response to CCK and D-CCK. CCK reliably elicited vigorous pyloric contraction as early as Day 1, characterized by an initial phasic peak followed by a lower chronic response. The magnitude of pyloric contraction, including both phasic and tonic components, increased markedly with increasing CCK dose at all ages. The minimum CCK dose required to elicit contraction increased with age. In addition, the latency of contraction increased with decreasing CCK dose at all ages. D-CCK elicited some contraction at 100 nM in day 1, 3 and 6 pyloric segments, but was ineffective by day 10. Atropine blocked the contractile response to carbachol, yet did not eliminate CCK elicited contraction at any age, suggesting that CCK does not act by a muscarinic receptor mechanism. (Supported by DDK-19302)

507.15

INTRAVENOUS BOMBESIN REDUCES FOOD INTAKE IN THE RAT. E.I. Yen*, J. Miesner*, J. Gibbs, A. Weller, and G.P. Smith. Bourne Lab., Dept. of Psychiatry, The NY Hosp-Cornell Med. Ctr., White Plains, NY 10605.

Intraperitoneal (ip) injections of bombesin (BBS; Gibbs et al, 1979) and mammalian BBS-like peptides (eg. GRP-10, DiPoala and Gibbs, 1985) reduce food intake in rats, suggesting that this peptide family may play a physiological role in satiety. We tested whether the intravenous (iv) infusion of BBS would produce similar effects. Methods: Adult male Sprague Dawley rats with chronic inferior vena cava catheters were maintained on solid food. At 1000, rats were food deprived; at 1050 they were given an iv infusion of BBS (4,8, or 16 ug-kg^{-1}) or equivolumetric (1 ml) vehicle over 10 min; at 1100, liquid food (BioServ, 40% v/v) was offered and intakes measured at intervals during a 150 min test. Results: BBS reduced intake in a dose-dependent fashion. Percent reductions at 30 and 150 min were:

Dose	30 min	150 min	p value
4	35	15	<0.07
8	42	24	<0.01
16	67	39	<0.001

Conclusion: Intravenous infusions of BBS produce large, dose-dependent reductions in food intake. Thus, a local site of action within the abdomen, accessed by the ip route, is not required for the satiety effect of exogenous BBS in the rat. Supported by NIH grant DK33248 (JG).

507.16

EFFECT OF PYLORECTOMY ON THE INHIBITION OF GASTRIC EMPTYING BY CHOLECYSTOKININ (CCK) Timothy H. Moran, Robert J. Crosby*, and Paul R. McHugh. Department of Psychiatry, Johns Hopkins University School of Medicine, Baltimore, MD 21205

Removal of a population of type A CCK receptors localized to the circular muscle layer of the pyloric sphincter by pylorectomy results in a significant attenuation of the ability of CCK to inhibit food intake. The present experiment addresses the role of CCK's inhibition of gastric emptying in the mediation of these results. The gastric emptying of 10 ml loads of saline (0.9% NaCl) and glucose (.125 gm/ml) from the rats stomach following IP injections of 2 or 8 µg/kg or saline vehicle was quantified by the serial test meal dye dilution technique. Following acquisition of this baseline data, rats were pylorectomized, surgically removing pyloric CCK receptors and surrounding tissue. Two weeks following the pylorectomy procedure, at a time when the inhibition of food intake by CCK is significantly attenuated, the effect of CCK on the gastric emptying of saline and glucose test loads was reassessed. The results showed that preoperatively, CCK inhibited the emptying of saline and glucose test meals in a dose dependent manner. Pylorectomy had no effect on CCK's ability to inhibit the emptying of saline test meals but attenuated the inhibition of the emptying of glucose by CCK. While demonstrating some role for pyloric CCK receptors in the inhibtion of gastric emptying by CCK, these results suggest that this inhibtion is complex and may depend upon multiple sites of action and mechanisms. (Supported by DDK19302)

507.17

CENTRAL ALLOXAN TREATMENT DECREASES THE INHIBITION OF FEEDING INDUCED BY NALOXONE AND CHOLECYSTOKININ IN RATS. D. Artune and R. J. Bodnar, Dept. of Psychology, Queens College, CUNY, Flushing, NY, 11367.

While peripheral alloxan destroys pancreatic beta cells and thereby produces diabetes, central alloxan (200 µg, ICV) reduces 2-deoxy-D-glucose (2DG) hyperphagia without altering hyperglycemia, presumably by altering either brain glucoreceptors or a glucoprivic control mechanism. The present study evaluated whether central alloxan altered the inhibition of intake of male rats deprived of food for the previous 24 h by the opiate receptor antagonist, naloxone (NAL: 0.01-10 mg/kg, IP) or the peripheral satiety peptide, cholecystokinin octapeptide (CCK: 1-8 µg/kg, IP). Both NAL and CCK significantly and dose-dependently inhibited intake at 2, but not at 24, h following reintroduction of food in vehicle-treated rats. Peak inhibition of food intake by NAL (10 mg/kg) was significantly reduced following alloxan (41% inhibition) vs. vehicle (62% inhibition) treatment. Peak inhibition of food intake by CCK (4 µg/kg) was significantly reduced following alloxan (33% inhibition) vs. vehicle (54% inhibition) treatment. Alloxan shifted the ED50 of CCK inhibition to the right, but only reduced the peakof inhibitory effect for NAL. These data indicate that central alloxan can interfere with neurochemical systems producing inhibitory (NAL and CCK) as well as stimulatory (2DG) effects upon food intake.

507.18

INTRAVENOUS ADMINISTRATION OF CHOLECYSTOKININ ANTAGONIST INCREASES FOOD INTAKE IN THE RAT. J.A. Miesner*, E.I. Yen*, J. Gibbs, A. Weller, and G.P. Smith. (SPON: M. Russ). E.W. Bourne Lab., Dept. of Psychiatry, The NY Hosp-Cornell Med Ctr., White Plains, NY 10605.

Intraperitoneal (ip) bolus injections of the highly potent and specific CCK antagonist MK 329 (Merck, Sharp, & Dohme) increase short-term food intake in rats (eg, Reidelberger and O'Rourke, 1987; Schneider et al, 1988). We tested whether the slow intravenous (iv) infusion of MK 329 would also increase food intake. Methods: Adult male Sprague Dawley rats (n=8) were surgically equipped with chronic inferior vena cava catheters. At 1000, rats were food deprived and an infusion of MK 329 (1 mg-kg-h^{-1}) or equivolumetric vehicle control, was begun. Liquid food (40% v/v BioServ) was offered at 1100 and consumption measured at intervals throughout a 2.5h test period, during which the iv infusion continued at the same rate. Results: MK 329 increased food intake during the first 15 min of the test (17% increase, $p<0.05$), at 30 min (23%, $p<0.03$), at 45 min (29%, $p<0.03$), and across the total test period (20%, $p<0.01$). Conclusion: Intravenous MK 329 produces a clear and consistent increase in food intake in rats; the size of this effect correlates well with effects reported previously utilizing the ip route. This result lends further support for a physiological role for endogenous CCK in satiety. Supported by USPHS NIMH grant 40010 (GPS).

507.19

EFFECTS OF CHOLECYSTOKININ, LiCl AND HYPERTONIC NaCl SOLUTION ON GASTRIC ACID SECRETION IN RATS. Loretta M. Flanagan, Joseph G. Verbalis and Edward M. Stricker. Departments of Behavioral Neuroscience and Medicine, University of Pittsburgh, Pittsburgh, PA 15260.

Treatments with cholecystokinin, LiCl and hypertonic NaCl solution each are known to decrease food intake, inhibit gastric motility, and stimulate oxytocin secretion in rats. Because the descending projections of the paraventricular nucleus of the hypothalamus (PVN) have been implicated in the control of feeding and gastric motility, it has been hypothesized that the PVN coordinates this array of responses to these treatments. One might further expect gastric acid secretion to be co-regulated with feeding and gastric motility because electrical stimulation of PVN is known to increase acid secretion. To test that hypothesis animals were fitted with gastric cannulae and acid secretion was measured hourly in awake, freely moving animals for 4 h after each treatment (n = 5-6 for each group). After injection of cholecystokinin (0.1, 1.0, 10 ug/kg, ip) there was a dose-related increase in gastric acid secretion (193%, 230%, 259% of control, respectively). In contrast, LiCl (0.75, 1.5, 3.0 mEq/kg, ip) had no consistent effect on acid secretion (122%, 119%, 75%), whereas osmotic dehydration (2 ml, 2 M NaCl, iv) caused an inhibition of acid output (43%). These data indicate that the effects of various treatments on gastric acid secretion do not correlate well with their effects on food intake, gastric motility, or pituitary oxytocin secretion.

507.20

SATIATION FOLLOWING INTRADUODENAL INFUSION OF INTRALIPID OCCURS PRIOR TO APPEARANCE OF [^{14}C]-INTRALIPID IN PLASMA. D. Greenberg, R. Kava*, Z. Wojnar* and M.R.C. Greenwood*. NY Hosp.-Cornell Med. Ctr. White Plains, NY 10605 and Dept. of Biology Vassar Coll. Poughkeepsie, NY 12601.

Duodenal infusions of 5 kcal of Intralipid (IL) in sham feeding rats significantly reduce intakes and elicit behaviors typical of satiety (Greenberg et al. 1985). To determine whether these effects are mediated at pre-absorptive sites we examined the time course for absorption of IL into the systemic circulation under test conditions identical to those for intake measures.

Rats (n=7) were fitted with gastric cannulas and duodenal and inferior vena cava catheters. Rats were given 1.6 uCi [^{14}C]-IL at a concentration of 0.5 kcal/ml. All [^{14}C]-IL was given in the first 2.2 ml of a total volume of 10ml IL. A total caloric load of 5 kcal was delivered at a rate of 0.44 kcal/min. The time course of the appearance of [^{14}C] in 100ul plasma is shown below.

Time of:	First significant difference	Maximal effect
Appearance in Plasma:	30 min	120 min
Intake:	12.5 min	40 min

Since the latency for inhibition of intake is significantly shorter than the latency for [^{14}C] in plasma, these results are evidence for a preabsorptive site of action for fat-induced satiety.

Supported by: The Weight Watchers Foundation, and NIH DK38757 (DG); AM26687 (MRCG).

508.1

EFFECTS OF DOI AND AMPHETAMINE ON TIME PERCEPTION IN RATS. R. Pastel, T. Raslear* & D. Shurtleff*. Dept. Medical Neuroscience, Walter Reed Army Institute of Research, Washington, DC 20307

Recently, our laboratory has demonstrated that very small doses (0.003 & 0.01 mg/kg) of 1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane (DOI), a 5HT-2 agonist, increase REM sleep in rats. Since 5HT-2 agonists have been reported to be hallucinogens, we were interested whether DOI might alter time perception in rats. In addition, we looked at the effects of amphetamine (AMP) which has been claimed by others to alter time perception in rats, but no dose-response studies have been reported. We used a temporal bisection procedure to obtain estimates of the perceptual midpoint of the light duration interval bounded by 1.0 and 2.5 sec and of the discriminability of those two stimuli.

No effect was found after low doses (0.01-0.1 mg/kg) of DOI. Both the 0.375 and 0.75 mg/kg doses appeared to increase the bisection point in a dose-dependent manner. Discriminability declined as the dose increased, and a bias in reporting "short" was seen after these doses of DOI. There were no apparent changes in the bisection point as a function of dose after AMP (0.75 and 1.5 mg/kg). Discriminability declined as the dose increased, but no response bias was seen. The highest doses of both AMP (3.0 mg/kg) and DOI (1.5 mg/kg) induced erratic behavior, so these doses were not analyzed.

508.2

SEXUAL BEHAVIOUR IN MALE RATS AND POSTEJACULATORY ULTRASOUNDS ARE DIFFERENTIALLY AFFECTED BY 8-OH-DPAT AND IDAZOXAN. B.Olivier, J.Mos* and J.van Logten*. CNS-Pharmacology, Duphar b.v., P.O.Box 2, 1380 AA Weesp, Holland.

8-OH-DPAT, a 5-HT_{1a} agonist, facilitates rat sexual behaviour in male rats as evidenced by decreased mounts and intromissions needed to reach ejaculations and a shortening of the refractory period following ejaculations. The α_2 antagonist idazoxan is thought to act differently by increasing motivation, reflected by increased mounting. The behavioural effects in moderately trained male rats of 8-OH-DPAT (0.1 and 0.4 mg/kg s.c.) and idazoxan (1 and 10 mg/kg i.p.) were studied as well as the postejaculatory ultrasounds emitted by rats during the refractory period.
Both drugs facilitated rat male sexual behaviour, but contrary to previous results idazoxan also shortened the latency to the first ejaculation. Only 8-OH-DPAT also reduced the refractory period. However, both drugs supressed ultrasounds (20-25 kHz) similarly. Ultrasounds of higher frequencies (>30 kHz) were differentially affected. On the basis of the pharmacological data we will speculate on the significance of ultrasonic communications by male rats after ejaculation.

508.3

ANALYSIS OF THE TEMPORAL STRUCTURE OF RAT PUP ULTRASOUNDS AND THE APPLICATION AS MODEL FOR ANXIETY DISORDERS. J.Mos*, J.van Logten*, A.J.Wolthuis*, G.van der Poel* and B.Olivier (SPON: J.Schipper). CNS-Pharmacology, Duphar b.v., P.O.Box 2, 1380 AA Weesp, Holland.

Rat pups emit ultrasounds of 35-50 kHz when they are separated from their mother and littermates. Several external factors influence this behaviour, e.g. temperature, odours, bedding as well as endogenous variables, like age and duration of separation.
In a number of experiments the temporal structure of these calls was investigated. Temperature of the test environment markedly changed the structure of ultrasounds i.e. more bursts of repeated calls were observed at low temperatures (18°C) whereas at 37°C more isolated fragmented calls were observed.
Serotonin reuptake blockers (fluvoxamine), 5-HT_{1a} agonists like buspirone and flesinoxan and benzodiazepines affect not only the number of ultrasounds, but also the distribution in time. Many drugs do not affect these calls. On the basis of these results it seems possible to classify differential actions of various drugs effective in different forms of anxiety disorders.

508.4

LOCAL INJECTION OF SEROTONIN INTO THE LATERAL HYPOTHALAMUS SUPPRESSES FOOD INTAKE. H.L. West, D.H. Schwartz and B.G. Hoebel. Department of Psychology, Princeton University, Princeton, NJ 08544-1010

Previous research suggests that serotonin exerts an inhibitory influence on feeding. Results using microdialysis demonstrated that extracellular serotonin increased in the perifornical region of the lateral hypothalamus (PFH) in conjunction with a meal[1] or injection of peripheral or local d-fenfluramine[2]. In the present study, the anorectic potency of lateral hypothalamic serotonin was investigated during the three-hour period following central injections in mildly food-deprived rats. Serotonin (6.3, 12.5* and 25* nmol) injected bilaterally into the PFH produced a dose-dependent reduction in food consumption at 15 and 30 minutes post-injection (* $p<0.02$). No differences in cumulative intake were detected after 60 minutes at any of the doses tested. The suppressive effect of serotonin (25 nmol) 30 minutes post-injection was found to be significantly attenuated by pretreatment with systemic injection of the serotonin antagonist metergoline (1 mg/kg), but not by the dopamine receptor antagonist haloperidol (0.25 mg/kg). These results support the hypothesis that serotonin transmission in the PFH contributes to the inhibition of feeding behavior.
[1]Schwartz, D.H., McClane, S., Hernandez, L. & Hoebel, B.G. (1989) Feeding increases extracellular serotonin in the lateral hypothalamus of the rat as measured by microdialysis. Brain Research, 479, 349-354.
[2]Schwartz, D., Hernandez. L. & Hoebel, B.G. (1989) Fenfluramine administered systemically or locally increases extracellular serotonin in the lateral hypothalamus as measured by microdialysis. Brain Research, 482, 261-270.

508.5

THE ROLE OF SEROTONIN RECEPTORS IN PRODUCING AN INVERTED U-SHAPE RESPONSE ON AGGRESSION. J.F.Bourg* and K.M.Kantak (SPON:J. Liederman). Lab of Behav. Neurosci., Dept. Psychol., Boston, MA 02215.

Serotonin (5-HT) is implicated in aggressive behavior in humans and animals. Pharmacologically, at least three distinct 5-HT receptor subtypes (5-HT-1a, 5-HT-1b, and 5-HT-2) have been identified to which different behavioral and neurophysiological functions have been ascribed. To elucidate seemingly contradictory data on the role of 5-HT in aggression, we have tested the effects of various 5-HT receptor agonists and antagonists on resident-offensive aggression in mice. The results suggest that there is an inverted U-shape function between 5-HT and aggression, with specific receptor subtypes having a differential role in producing this function. When 5-HT activity is reduced, as with pre-treatment with PCPA or mianserin, aggression is below normal levels. When 5-HT-1a or 1b agonists, such as mCPP, PAPP, 5MT or quipazine, are administered there is also a decrease in aggressive behavior. That 5-HT-2 stimulation by DOB results in a decrease in normal levels of aggression when administered alone and an increase in aggressive behavior after depletion of endogenous 5-HT could suggest that 5-HT-1 receptors mediate 5-HT elicited inhibition of aggression, whereas, 5-HT-2 receptors mediate 5-HT elicited excitation of aggression only when 5-HT-1 receptor activation is inhibited. Increases or decreases in the frequency of aggressive behavior are a result of an imbalance of receptor subtype stimulation.

508.6

ACUTE DECREASED PLATELET SEROTONIN AND ATTENUATION OF AUTONOMIC ACTIVITY IN THE TRANSCENDENTAL MEDITATION PROGRAM (TM). D. A. Hill[1]*, R.K. Wallace[1], K. G. Walton[1], and L. R. Meyerson[2]. [1]Neuroscience Dpt., Maharishi International Univ., Fairfield, IA 52556, and [2]American Cyanamid Company, Ramapo College, Mahwah, NJ 07430.

TM is a widely practiced mental technique that results in improved health and adaptation to stress. Previous research has documented an acute attenuation of the SNS during TM, but no direct investigation of acute PNS effects has been reported. Also the acute effects of TM on the monoamines, serotonin (5-HT) in particular, remain to be clarified. We undertook a study of heart rate variability (HRV) in response to respiration (respiratory sinus arrhythymia) as an indirect measure of cardiac vagal tone during TM. Skin Resistance (SR) was employed as an indicator of SNS tone. The results were that during TM, particularly at times of spontaneous respiratory apneusis that are known to mark the subjective experience of "transcending", HRV was almost absent and SR showed no change. These results indicate that both PNS and SNS tone were attenuated. In a second experiment we measured changes in platelet 5-HT levels (HPLC-ECD), plasma tryptophan (spectrofluorimetry), and prolactin (PL) and lutenizing hormone (LH) by RIA and alpha-1-acid glycoprotein (AGP, a modulator of 5-HT uptake) by radial immunodiffusion assay, in 25 male subjects before and after TM in relation to biographical variables and Type A Behavior (TAB). Significant reductions in platelet 5-HT ($t=4.37$, $p<0.001$) and PL ($t=4.47$, $p<0.001$) were observed with no changes in tryptophan or AGP. Years practicing TM was negatively correlated ($r=-0.5$, $P<0.01$) with post TM PL levels and positively correlated ($r=0.51$, $P<0.02$) with the 5-HT change score. These results support the hypothesis of acutely reduced central and peripheral 5-HT activity during TM practice. There was a positive correlation ($r=0.49$, $P<0.05$) between the "Hard-Driving and Competitive" subscale of TAB and PL post TM indicating a possible increase in central 5-HT activity with TAB. In another group tested comparing smokers to non smokers, a 34% increase in AGP was observed in the smokers ($t=2.67$, $P=0.012$).

508.7

ANTIDEPRESSANT-LIKE EFFECTS OF THE 5HT1A AGONISTS BUSPIRONE, GEPIRONE, 8OHDPAT AND 5MEODMT IN RATS ON THE DRL 72 SEC SCHEDULE: DIFFERENTIAL BLOCKADE BY METHYSERGIDE AND PURPORTED 5HT1A ANTAGONISTS Timothy H. Hand, Gerard J Marek, David C. Jolly and Lewis S. Seiden, Dept. Pharmacol. Physiol. Sci., Univ. Chicago, Chicago IL 60637

The 5HT1A agonists 8OHDPAT and 5MeODMT exert antidepressant-like actions on the DRL 72 sec schedule (increased reinforcement rate). Buspirone, gepirone and ipsapirone are also potent 5HT1A agonists. Since buspirone and gepirone have recently been shown to act as antidepressants in open trials, we evaluated their effects on the DRL schedule. Buspirone and gepirone (1.25 - 5 mg/kg) produced increases in reinforcement rate. Ipsapirone did so only at 20 mg/kg. 8OHDPAT and 5MeODMT also showed AD-like effects, as previously shown. The purported 5HT1A antagonists BMY 7378 and NAN-190 antagonized the AD-like effects of buspirone and gepirone but were ineffective against 8OHDPAT or 5MeODMT. The opposite pattern was observed with methysergide, which antagonized the effects of 8OHDPAT and 5MeODMT but which was ineffective against buspirone and gepirone. (-)Propranolol, an antagonist at 5HT1A and B adrenergic receptors, gave mixed results. It is concluded that 1) consistent with their clinical activity, buspirone and gepirone act as antidepressants on a behavioral screen, 2) their mode of action differs from that of 5MeODMT and 8OHDPAT, and 3) there may be multiple populations of 5HT1A receptors. (Supported by MH 11191 and 10562 to LSS)

508.8

POTENTIATED 5-HYDROXYTRYPTOPHAN (5-HTP) RESPONSE SUPPRESSION FOLLOWING 5,7-DIHYDROXYTRYPTAMINE (5,7-DHT) RAPHE LESIONS IN RATS TESTED IN A MODEL OF DEPRESSION. E.A. Engleman*, J.N. Hingtgen, F.C. Zhou, J.M. Murphy, and M.H. Aprison. Depts. of Psychiatry; Biochem.; Anatomy; Program in Medical Neurobiology; Inst. of Psychiat. Res., Indiana Univ. Sch. of Med., Indianapolis, IN 46202.

In an effort to better define the neurochemical/behavioral interrelationships involved in affective disorders, Aprison and Hingtgen have developed the hypersensitive postsynaptic serotonin (5-HT) receptor theory of depression. A behavioral model was developed in which rats, trained to press a lever for milk reinforcement on a VI 1' schedule, showed a period of response suppression after IP injections of D,L-5-HTP. The present study addresses the effects of central 5-HT depletion via intraraphe injections (25 mg/ml into 6 sites) of 5,7-DHT on 5-HTP induced behavioral suppression. Two weeks after such chemical lesioning, the rats exhibited an increased mean 5-HTP suppression response that was greater than the nonlesioned controls. HPLC assay of monoamine contents showed that rats with lesions had an average decrease in frontal cortical 5-HT of 80% compared to the nonlesioned animals. Less depletion was observed in hypothalamus and brain stem sections. This 5,7-DHT method of reducing cerebral 5-HT and potentiating the 5-HTP behavioral suppressions, supports our earlier work with an animal model of depression. (Supported in part by Indiana Dept. of Mental Health).

508.9

SEROTONERGIC INVOLVEMENT IN DOPAMINE-DEPENDENT CATALEPTIC RESPONSES IN THE RAT. B.S. Neal, I. Lucki and J.N. Joyce, Departments of Pharmacology and Psychiatry, University of Pennsylvania School of Medicine, Philadelphia, PA 19104.

Antipsychotic-induced catalepsy is primarily associated with dopamine (DA) receptor blockade. In addition, central serotonergic function may have a significant influence upon this type of catalepsy. Our aim was to examine the ability of several dopaminergic compounds, including selective DA D1 (SKF38393) and DA D2 (quinpirole) receptor agonists, to block catalepsy. We also examined the ability of 5-HT agonists to affect antipsychotic-induced catalepsy, using agents which act selectively at the various 5-HT receptors. The cataleptic effects of haloperidol (2 mg/kg, s.c.) were tested in male, Sprague-Dawley rats, by placing the rats' forelimbs over a horizontal bar 10 cm above the ground.

The dopaminergic compounds, apomorphine (0.1-3.2 mg/kg), d-amphetamine (1.0-5.6 mg/kg), and quinpirole (0.1-3.2 mg/kg) were each able, at certain doses, to reverse haloperidol-induced catalepsy. SKF38393 (1.0-32 mg/kg) had no significant effect.

The following serotonergic compounds, at certain doses, also reversed catalepsy: 8-hydroxy-DPAT (0.01-1.0 mg/kg), buspirone (0.1-3.2 mg/kg), TFMPP (1.0-5.6 mg/kg) and DOB (0.1-3.2 mg/kg). Comparisons between dopaminergic and serotonergic agents were also conducted by examining shifts in the dose-effect curves for haloperidol-induced catalepsy.

These results suggest that not only is antipsychotic-induced catalepsy caused by DA D2 receptor blockade, but there is also a significant serotonergic influence, involving more than one of the 5-HT receptor subtypes. Studies are currently underway to further elucidate how and where 5-HT is acting to mediate catalepsy.

This research was supported by U.S.P.H.S. grants MH14654 (B.S.N.), MH43852 (J.N.J.), MH36262 and GM34781 (I.L.).

508.10

AN ANALYSIS OF THE INCREASED SOCIAL INTERACTION PRODUCED BY THE INTRA-AMYGDALA INJECTION OF VARIOUS 5-HT3 RECEPTOR ANTAGONISTS IN RATS

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Both systemic and intra-amygdala injections of the 5-HT_3 receptor antagonists GR38032F, MDL72222 (MDL), ICS205-930 (ICS), BRL43694 (BRL) & GR65630A in the rat increase social interaction (SI) under conditions of maximal suppression, ie high light/unfamiliar (HLU). After intra-amygdala injections of GR38032F (100ng), MDL (100ng), ICS (100ng), BRL (10ng) & GR65630A (10ng) the predominant behavioural changes were increased sniffing, following & grooming of the untreated partner rats. Individual bout frequency & duration were also increased. These behaviours were similar to those induced in control rats by environmental manipulation, ie low light/familar (LLF) compared to HLU conditions, indicating that they reflect a reduced anxiety state. Conversely, the amygdala injections of 5-HT & 2-ME-5-HT (both 10,000ng) reduced SI under the LLF condition, characterised by reduced bout frequency & duration.

These findings provide evidence that the disinhibitory effects of 5-HT_3 receptor antagonists in the rat SI test are mediated at least in part, via the amygdala.

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508.11

COCAINE-INDUCED LOCOMOTOR ACTIVITY: A MODULATORY ROLE FOR SEROTONIN. M.E.A. Reith, H.K. Kramer*, H.L. Wiener*, and C. T. Fischette§. Center for Neurochemistry, Ward's Island, New York, NY 10035 and §Pfizer Pharmaceuticals , N.Y. 10017.

Although cocaine potently inhibits serotonin uptake, the contribution of this property to cocaine's central activity is unclear. In the present study, serotonergic systems were activated by treatment of C57 BL/6ByJ mice with sertraline (SER), a potent and selective serotonin uptake inhibitor, 60 min prior to cocaine (COC). Compared with placebo, SER (1-32 mg/kg, i.p.) did not change the locomotor activity measured for 50 min after injection of COC (25 mg/kg i.p.) or saline in male or female C57 mice. Pretreatment with 8 mg/kg of SER or with placebo resulted in identical dose-response curves for COC (10-40 mg/kg), indicating that the lack of effect of SER was not due to a ceiling effect. Although SER did not potentiate or attenuate the overall locomotor response to COC, there was a shift in the response towards later times at 16 and 32 mg/kg of SER, both in male and female mice. This was probably not due to a pharmacokinetic interaction between the compounds, because pretreatment with SER (16 & 32 mg/kg) did not affect the plasma and brain concentration of COC measured (by HPLC) 12 min after injection of COC (25 mg/kg) when brain levels of COC were maximal. The SER-induced delay of the locomotor response to COC is consonant with other pieces of evidence pointing to an inhibitory modulation by serotonin of COC's locomotor and rewarding effects. (Support:Pfizer Pharmac. #88-N-0123)

508.12

STRESS AS THE CAUSE OF SEROTONERGIC DYSFUNCTION IN MENTAL DISORDERS: SUPPORT FROM CORRELATES OF SEROTONIN TURNOVER IN NORMAL SUBJECTS. K.G. Walton,[1,2] P. Gelderloos,[3]* N.D.C. Pugh,[1,2]* P. McRae,[3]* P. Goddard,[3]* C. MacLean[1,2]* and D. Levitsky[1,2]*. [1]Neurochemistry Laboratory, Departments of [2]Chemistry and [3]Psychology, Maharishi International University, Fairfield, IA 52556.

Central serotonergic activity appears reduced in a variety of personality and affective disorders. Reduced serotonin turnover has also been reported in depressed subjects, even on the whole-body level. We investigated excretion of the serotonin metabolite, 5-hydroxyindoleacetic acid (5-HIAA), an accessible indicator of whole-body serotonin turnover, in relation to standard tests of personality and affect in normal subjects. In the first study, 5-HIAA excretion in 33 women students (**but not men**) correlated negatively with trait anxiety and depression ($r = -.47$ and $-.51$, respectively, $P < .005$). In a second study, 5-HIAA correlated negatively with aggression ($r = -.43$, $P < .005$) and impulsiveness ($r = -.28$, $P < .05$) **only in men.** These correlations and sex differences persisted in a low-stress group. The low-stress group also showed higher night-time serotonin turnover ($P < .00005$, $df = 52$) and a day-night difference in turnover (night higher) not found in the average-stress group. These sex differences in correlates of serotonin turnover parallel the prevalence of mental disorders in the population as a whole; anxiety and affective disorders are twice as frequent in women whereas in men antisocial personality disorders are four times more frequent. This correspondence between sex-related differences in mental disorders and the psychological correlates of serotonin turnover in both low- and average-stress students suggests involvement of the same physiological mechanism. Based on animal studies showing reduced serotonergic activity in cases of unavoidable chronic or extreme stress, we conclude accumulated stress is the likely cause of reduced serotonergic activity in a number of mental disorders.

508.13

SEROTONERGIC DORSAL RAPHE UNIT ACTIVITY RELATED TO FEEDING/GROOMING BEHAVIORS IN CATS. L.E. Ribeiro-do-Valle, C.A. Fornal, W.J. Litto and B. L. Jacobs. Prog. Neurosci., Dept. of Psychol., Princeton Univ., Princeton, NJ 08544.

The discharge activity of central serotonergic neurons has been shown so far to basically vary in association with behavioral state. We report here on a subgroup of dorsal raphe nucleus (DRN) serotonergic neurons that dramatically change their activity in relation to feeding/grooming behaviors. DRN serotonergic cells were recorded using methods described previously (Fornal et al., Exp. Neurol. 98: 338-403, 1987). Single-unit activity was monitored during spontaneous waking behaviors and during sleep. In addition to highly regular firing DRN serotonergic neurons, we found cells that discharged in a somewhat regular manner during quiet waking (2.0±0.4 spikes/s, N=9, 7 cats), and were completely off during REM sleep and in response to systemic administration of serotonin agonist drugs. These cells displayed a firing rate of 5.3±1.1 spikes/s (N=7) during feeding-related movements (e.g. licking) and 5.8±1.4 spikes/s (N=6) during grooming-related movements. During other active waking movements these cells exhibited irregular discharge (often pausing), and their firing rate was similar to or less than that during quiet waking. Our results indicated that serotonergic neurons may modulate target structures in association with specific behaviors, as well as, the general level of behavioral arousal.

508.14

EFFECTS OF 8-HYDROXY-2-(DI-N-PROPYLAMINO) TETRALIN (8-OH-DPAT) ON FOOD INTAKE AND SEROTONERGIC DORSAL RAPHE UNIT ACTIVITY IN BEHAVING CATS. B.L. Jacobs, C.A. Fornal, L.E. Ribeiro-do-Valle, W.J. Litto, and L.O. Wilkinson. Prog. Neurosci., Dept. of Psychol., Princeton Univ., Princeton, NJ 08544.

Recent studies have shown that selective 5-HT$_{1A}$ agonists, such as 8-OH-DPAT, increase food consumption in rats. This action is thought to be mediated via an inhibition of midbrain serotonergic neurons as a result of direct activation of somatodendritic autoreceptors. We examined the effects of 8-OH-DPAT on food intake and single-unit activity of serotonergic dorsal raphe nucleus (DRN) neurons. Cats fed ad libitum were administered 8-OH-DPAT (10 - 1,000 ug/kg, s.c.) or saline vehicle in a randomized design, and food intake was monitored over the next 24 hrs. A separate group of cats was administered 8-OH-DPAT (10, 50, or 250 ug/kg, s.c.) and unit activity was monitored prior to, and for 6 hrs following injection. Low doses of 8-OH-DPAT (50 ug/kg) consistently produced hyperphagia, whereas higher doses produced an initial suppression (0.5 - 1.5 hrs), followed by an increase in food intake. 8-OH-DPAT completely inhibited unit activity for approximately 1.5 and 3.0 hrs following 50 and 250 ug/kg 8-OH-DPAT, respectively. These data suggest that preferential activation of serotonin somatodendritic autoreceptors produces an increase in food intake in free feeding cats.

INVERTEBRATE LEARNING AND BEHAVIOR IV

509.1

CELLULAR ANALOGUE OF A CLASSICAL CONDITIONING PROTOCOL PRODUCES LONG-TERM CHANGES IN SENSORY TO MOTOR NEURON SYNAPSES IN *APLYSIA*. D.V. Buonomano and J.H. Byrne. Dept. of Neurobiology and Anatomy, Univ. of Texas Med. Sch., Houston, TX 77025.

Previous work has demonstrated that a cellular analogue of a differential classical conditioning protocol produces a short-term (min) form of associative plasticity termed activity-dependent neuromodulation in the connections between sensory neurons (SN) and motor neurons (MN) in *Aplysia* (Hawkins et al., 1983; Walters and Byrne, 1983). EPSPs produced by SNs whose activity was paired with a reinforcing stimulus were significantly larger than unpaired controls. We have extended this observation by examining long-term (24 hr) enhancement of EPSPs produced by a similar training protocol.

Two SNs in the pleural ganglia, which synapsed onto a common MN in the pedal ganglia, were fired 3 times with a 5 sec interspike interval to establish baseline EPSPs. Training consisted of activating a SN (SN+) with a 10Hz, 1 sec train of depolarizing pulses 400 msec before the onset of a 50Hz, 500 msec electric shock to nerve P9 or P8. The second SN (SN-) was activated with the same parameters either 2.5 min before or after nerve shock (counterbalanced). There were a total of 5 training trials with an interval of 5 min. The short-term effect of training was examined by testing the connections 5 min after training and the long-term effects were examined by retesting the connections 24 hr later. The amplitudes of EPSPs of both the 5 min and 24 hr tests were normalized to their baseline values.

There was a significant short-term associative effect ($p<0.02, n=10$) determined by comparing the EPSPs produced by the of SN+ group (210%±26) to those produced by the SN- group (160%±13) 5 min after training. Moreover, 24 hr after training there was a significant ($p<0.05, n=10$) enhancement of the EPSPs produced by the SN+ group (420%±70) compared to those produced by the SN- group (290%±48). Twenty-four hrs after training the EPSPs of both the SN+ and SN- groups were larger than their 5 min values, but the relative proportion of associative to nonassociative plasticity was similar. This study establishes for the first time the existence of long-term (24 hr) associative plasticity of synaptic connections in *Aplysia* and will permit further studies on the mechanisms underlying the induction, storage and retrieval of long-term associative memories.

509.2

LONG-TERM (24 HR) ENHANCEMENT OF THE SENSORY-MOTOR CONNECTION MEDIATING TAIL WITHDRAWAL REFLEX IN *APLYSIA* IS PRODUCED BY NERVE STIMULATION, AN *IN VITRO* ANALOGUE OF SENSITIZATION TRAINING. J.R. Goldsmith* and J.H. Byrne. (SPON: S.D. PAINTER) Dept. of Neurobiology & Anatomy, University of Texas Medical School, Houston, TX 77225.

An *in vitro* analogue of sensitization training was used to examine both short-term (min) and long-term (24 hr) changes in the monosynaptic connections between sensory (SN) and motor neurons (MN) in the pleural-pedal ganglia, which mediate the tail withdrawal reflex. The analogue was designed to simulate closely both the intensity and timing of the shocks delivered to the body wall of the animal during sensitization training (Scholz & Byrne, 1987).

Training consisted of 4 trains of shocks delivered to nerves P7, P8 and P9 over a 1.5 hr period. The connections between the SN and MN were examined before, 5 min after, and 24 hr after the training procedure by firing a SN with a brief intracellular depolarizing pulse and recording the monosynaptic EPSP in the MN. Motor neurons were hyperpolarized by 30 mV during each test. After the first post-test, electrodes were removed and cells adjacent to the SN and MN were injected with fast green to aid in later identification. The ganglia were maintained in organ culture for the 24 hr period between recordings.

The mean amplitude of the EPSP recorded 5 min after training was significantly larger than baseline (10.9±1.3 mV vs. 4.2±0.5 mV respectively; $p<0.001$, $n=8$). Moreover, the mean amplitude of the EPSP recorded 24 hr after training was still significantly larger (8.9±1.3 mV; $p<0.005$, $n=8$) than baseline. This long-term increase in EPSP amplitude did not appear to be the result of changes in the MN. Resting membrane potentials of the MNs recorded immediately after training and 24 hr after training were not significantly different from those recorded prior to training. The mean input resistance of the MN (measured while the cell was hyperpolarized by 30 mV) 5 min after training was not significantly different from the pre-test measure (6.9±0.6 MΩ vs. 8.0±0.6 MΩ respectively, $n=8$). It was, however, significantly decreased (4.8±0.5 MΩ, $p<0.001$, $n=8$) 24 hr after training. Thus, the EPSPs measured at 24 hr were facilitated even though the input resistance of the MNs was decreased.

These results indicate that a neural analogue of behavioral sensitization training is capable of producing both significant short-term and long-term enhancement of the sensory-motor connections responsible for mediating the tail withdrawal reflex. This simplified system may serve as a useful model with which to explore further the mechanisms of long-term facilitation.

509.3

BIOPHYSICAL AND PROTEIN CHANGES PRODUCED IN PLEURAL SENSORY NEURONS OF *APLYSIA* BY ELECTRICAL STIMULATION, AN *IN VITRO* ANALOGUE OF SENSITIZATION TRAINING. F. Noel*, K.P.Scholz, A. Eskin and J.H. Byrne. Dept. of Neurobiol. & Anat., Univ. of Tex. Med. Sch., Houston, TX, 77225, and Dept. of Biochem., Univ. of Houston, TX.

Stimuli applied to the tail of *Aplysia* activate sensory neurons in the pleural ganglion, which mediate a withdrawal reflex of the tail and siphon. Application of 4 trains of noxious stimuli to one side of the animal over a 1.5 hr period produces both long-term (24 hr) sensitization of the reflex elicited by stimuli to the trained side and long-term reduction of net outward currents in sensory neurons from the trained side (Scholz and Byrne, 1987).

An *in vitro* analogue of the training procedure was developed in which we delivered 4 trains of shocks to the peripheral nerves of isolated pleural-pedal ganglia. As was the case for behavioral training, nerve stimulation led to long-term reduction of net outward currents in sensory neurons from the stimulated side compared to those of the contralateral (unstimulated) control ($n=10$).

In order to study proteins that may be responsible for the induction of long-term sensitization, we studied the effects of nerve stimulation on the incorporation of amino acids into proteins of sensory neurons. During nerve stimulation, both experimental and control ganglia were exposed to ^{35}S methionine (0.15 mCi/ml). At the end of stimulation, experimental and matched control clusters of sensory cells were removed and the samples were run on 2D PAGE. Eight proteins appeared to be changed by electrical stimulation, based on the criteria that changes were observed in at least 5 out of 7 experiments. Incorporation of amino acid was decreased in 5 proteins and increased in 3 proteins. In addition, we compared the effects of electrical stimulation with those of 1.5 hr application of 5-HT ($5x10^{-6}$ M). At least 2 of the proteins affected by electrical stimulation were changed in a similar way by 5-HT. Moreover, 2 proteins affected by electrical stimulation did not appear to be affected by 5-HT.

These results demonstrate that electrical nerve stimulation leading to long-term biophysical changes in the sensory neurons also leads to the alteration of specific proteins during the period of stimulation. Moreover, the effects of electrical stimulation may be mediated at least in part by 5-HT.

509.4

INTRACELLULAR INJECTION OF cAMP PRODUCES A LONG-TERM (24 HR) INCREASE IN THE NUMBER OF VARICOSITIES IN PLEURAL SENSORY NEURONS OF *APLYSIA*. F. Nazif*, J.H. Byrne, L.J. Cleary. Dept. Neurobiology and Anatomy, Univ. Texas Med. School, Houston, Texas, 77225.

Long-term sensitization has been correlated with changes in synaptic efficacy (Frost et al., 1985), membrane currents (Scholz and Byrne, 1987) and cell structure (Bailey and Chen, 1988) in sensory neurons mediating defensive withdrawal reflexes. Since the intracellular injection of cAMP is sufficient to mimic the effects of sensitization on membrane currents (Scholz and Byrne, 1988), we were interested in determining whether it was sufficient to alter cell morphology as well.

Either cAMP or 5'-AMP (200 mM) was injected by iontophoresis into pleural sensory neurons. Approximately 22 h later, the same neurons were injected with HRP by pressure pulses and incubated for another 2-4 h. The number of varicosities in branches within the neuropil of the pleural ganglion was greater in neurons injected with cAMP than in their contralateral controls injected with 5'-AMP (41 ± 9 SEM vs 24 ± 7 SEM; $p<0.02$, $n= 7$). Injections of sensory neurons with HRP alone suggest that neither the iontophoretic procedure itself nor overnight incubation contributed directly to the effect of cAMP.

Injection of cAMP appears to produce changes in sensory neuron morphology that are consistent with changes that occur as a result of long-term sensitization. By increasing the number of varicosities, the sensory neurons are presumably increasing the number of synaptic contacts they make with follower interneurons in the pleural ganglion (Cleary and Byrne, 1986). However, behavioral sensitization has multiple effects on neuron morphology. Further experiments will be required to determine whether all of the changes produced by behavioral sensitization are mimicked by injection of cAMP.

509.5

CHARACTERIZATION OF PROTEIN PHOSPHATASES IN *APLYSIA*.
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Protein phosphorylation-dephosphorylation is widely recognized as an important mechanism for cellular regulation. The phosphorylation state of a given protein reflects the activities of both protein kinases and protein phosphatases (PrPs). In *Aplysia*, cAMP-dependent protein kinase plays a key role in the induction and the maintenance of sensitization (Kandel, E. R. and Schwartz, J. H., *Science*, **218**, 433, 1982). In contrast, the role of PrPs in this process remains unclear. We have observed that okadaic acid, a specific inhibitor of protein dephosphorylation, rapidly altered membrane currents in voltage clamped pleural sensory cells. To further characterize the PrPs involved, we have assayed extracts of pleural sensory neurons (SN), abdominal ganglion (AG), and buccal muscle (BM) using ^{32}P-labelled phosphorylase *a* as a substrate. The specific activity (nmol phosphate released/min/mg protein) of PrP in these tissues was SN>AG>BM, ranging from 5.0 (SN) to 0.9 (BM). PrP activity, with K_m for phosphorylase *a* of approx. 10 μM, was increased by extensive dilution of these tissues. Tissue PrP activities were inhibited 60-80% by the addition of inhibitor-2 (I_2), a specific protein inhibitor of type-1 PrP. These results demonstrate the presence of type-1 (I_2-sensitive) and type-2A (I_2-insensitive) PrPs in *Aplysia*, with characteristics similar to PrPs in mammalian tissues. This study represents the first step towards characterizing *Aplysia* PrPs and investigating whether PrP activity is altered by sensitizing stimuli.

509.6

DIFFERENTIAL EFFECTS OF THE PEPTIDE BUCCALIN AND SEROTONIN ON MEMBRANE CURRENTS, ACTION POTENTIAL DURATION, AND EXCITABILITY IN PLEURAL SENSORY NEURONS OF *APLYSIA*.
J.L. Raymond, E.E. Shulman*, D.A. Baxter, L.J. Cleary and J.H. Byrne. Department of Neurobiology and Anatomy, University of Texas Medical School, Houston, TX 77225.

Buccalin is a neuropeptide isolated from the buccal mass of *Aplysia* (Cropper et al., 1988). Since it is distributed throughout the CNS and affects membrane properties of sensory neurons (SNs) in the cerebral ganglion (Rosen et al., 1989), we looked for effects of buccalin on tail SNs located in the pleural ganglion.

Clusters of SNs from the pleural ganglion were isolated and maintained at 20°C in ASW. Spike duration was determined by eliciting single spikes with 3 msec depolarizing current pulses, and excitability was measured as the number of spikes elicited by a 1 sec depolarizing current pulse. With 3 and 5 nA 1 sec pulses, buccalin (50 μM) applied to the static bath significantly increased excitability (n=9). In the same preparations, buccalin produced a modest increase in spike duration that was not statistically significant. When 5-HT, which increases both excitability and spike duration, was subsequently applied, spike duration increased dramatically, but there was no further significant increase in excitability.

In tail SNs, the effect of 5-HT on excitability is due primarily to a cAMP-dependent decrease of the S-current ($I_{k,s}$), whereas its effect on spike duration is primarily the result of a cAMP-independent modulation of the delayed K^+ current ($I_{k,v}$). In order to determine which currents might be mediating the effect of buccalin, two-electrode voltage clamp experiments were performed. At 15°C, buccalin decreased an outward current similar to $I_{k,s}$ but did not have the same effect on $I_{k,v}$ as 5-HT.

Modulation of multiple membrane currents and subcellular processes in the pleural SNs contributes to the regulation of synaptic efficacy and learning in *Aplysia*. The SNs are modulated by a number of transmitters, each of which effects a specific subset of processes. This study suggests that, like 5-HT, buccalin acts to reduce $I_{k,s}$, resulting in an increase in excitability; but unlike 5-HT, buccalin does not significantly increase spike duration.

509.7

DIFFERENTIAL EFFECTS OF SEROTONIN, SCP$_B$ AND FMRFAMIDE ON PROCESSES CONTRIBUTING TO PRESYNAPTIC FACILITATION IN SENSORY NEURONS OF *APLYSIA*.
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Previous studies have indicated that multiple processes contribute to synaptic facilitation of sensory to follower neuron connections mediating defensive reflex responses in *Aplysia*. In addition to spike broadening, a process referred to as mobilization of transmitter has recently been proposed (Gingrich and Byrne 1985; Gingrich et al. 1988; Hochner et al. 1986). In order to further examine mobilization and its regulation, we have compared the effects of small cardioactive peptide (SCP$_B$), serotonin (5-HT) and FMRFamide on processes contributing to facilitation of EPSPs in depressed sensory-motor synapses.

Ganglia were pretreated with TEA (100 mM) in order to broaden the sensory neuron spikes into a range of spike durations for which additional broadening alone has little or no effect on release. Under this condition, changes in release are due primarily to mobilization. Single action potentials were evoked in LE sensory neurons at 60 sec intervals and the resultant EPSPs were monitored in follower neurons.

Serotonin (25 μM) produced two effects, spike broadening and a robust enhancement of the EPSP. In contrast, SCP$_B$ (4 μM) produced only spike broadening, with little or no increase in EPSP amplitude. In addition, 5-HT greatly increased the initial slope of the EPSP (indicative of enhanced mobilization), while SCP$_B$ did not. The inability of SCP$_B$ to enhance release at TEA-treated synapses was not due to unresponsive cells, since 5-HT was able to enhance the EPSPs from sensory neurons previously exposed to SCP$_B$. These results suggest that 5-HT, but not SCP$_B$, can produce mobilization of transmitter. Finally, we are currently exploring the antagonistic actions of FMRFamide on the dual processes of facilitation. Preliminary findings indicate that FMRFamide (15-30 μM) produced a modest, often transient narrowing of the action potential and a concomitant decrease in the EPSP amplitude, both in the absence and presence of SCP$_B$ or 5-HT.

509.8

LOCALIZATION OF 5-HT CONTAINING NEURONS WHICH MODULATE HERMISSENDA TYPE B CELL EXCITABILITY.
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Previous research indicates that 5-HT contributes to the modulation of Hermissenda (H.c) Type B cell excitability by associative learning. However, the location and functional significance of 5-HT containing neurons within the H.C. CNS which may participate in learning has not, until now, been determined.

The anatomical distribution and location of putative 5-HT neurons was mapped: 1) in whole-mounted CNSs using 5-HT immunocytochemistry, and 2) in vivo, by labelling with 5,7-diHT, following chronic administration of (and recovery from) this 5-HT neurotoxin. Electrophysiological recordings were obtained from cells within two small bi-lateral clusters in the cerebropleural ganglia (cpg), just lateral to the cpg commissure. Stimulation of cells (n=18) in either cluster produced a slow depolarization (0.5-2.7 mV) of Type B photoreceptors, often accompanied by a conductance decrease. 5-HT neurons were, in turn, synaptically inhibited by visual stimulation and by stimulation of caudal hair cells. Release of 5-HT from these neurons following pairings of light and rotation may contribute to associative-learning-produced changes in B cells.

509.9

EFFECT OF DOWN REGULATION OF PROTEIN KINASE C ON SHORT-TERM ENHANCEMENT OF GENERATOR POTENTIALS IN HERMISSENDA PRODUCED BY LIGHT AND 5-HT.
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Light paired with 5-HT produces both a short and long-term enhancement of the amplitude of light-evoked generator potentials of identified B photoreceptors. Previous results have shown that the application of protein kinase C (PKC) inhibitors (H-7 & sphingosine) prior to the presentation of light and 5-HT blocks the short-term enhancement of light-evoked generator potentials (Forrester and Crow, 1988; 1989). However, since both H-7 and sphingosine are reported to inhibit several protein kinases the contribution of PKC to the enhanced light response has not been determined. We now present evidence that down regulation of PKC blocks or reduces the short-term enhancement of light responses produced by pairing light with 5-HT.

Incubation of isolated nervous systems and semi-intact preparations with TPA (10^{-6}M or 10^{-7}M) for 6, 8 or 16 hrs. reduced PKC activity in both the membrane-associated fraction and cytoplasmic fraction. Pretreatment of nervous systems with TPA blocked or reduced the enhanced generator potentials produced by light and 5-HT. After incubation of nervous systems (N = 13) for 6 hrs. in 10^{-6}M TPA, the application of 5-HT (10^{-4}M) produced a non-significant increase in the steady state or plateau phase of light evoked generator potentials (mean increase = 6.5% of baseline control). In contrast to the effect of TPA incubations, pretreatment for 6 hrs. with an inactive phorbol ester (4α-phorbol) did not block the light-5-HT enhancement of the plateau phase of light evoked generator potentials (mean increase = 29.6% of baseline control; $P<.05$). These results indicate that PKC activation contributes to the induction of short-term plasticity of identified B-photoreceptors produced by light and 5-HT.

509.10

KINASE INHIBITORS DO NOT REVERSE SHORT - OR LONG-TERM ENHANCEMENT OF LIGHT RESPONSES IN IDENTIFIED HERMISSENDA B-PHOTORECEPTORS.
J. Forrester* and T. Crow (SPON: R. Nudo). Dept. of Neurobiology and Anatomy, The University of Texas Medical School, Houston, TX 77225.

Sphingosine and H-7, inhibitors of protein kinase C (PKC), have been recently shown to prevent both TPA and 5-HT induced short-term enhancement of light-evoked generator potentials of identified B-photoreceptors in Hermissenda (Forrester and Crow, 1988). Additional evidence supporting a role for PKC in this example of plasticity comes from studies showing that down regulation of PKC blocks or reduces the short-term light-5-HT enhancement of generator potentials (Crow et al., 1989). We now report that sphingosine and H-7 do not reverse the short-or long-term light-5-HT enhancement when added after the enhancement of the generator potentials has already taken place.

To examine the possible contribution of PKC to the expression of enhanced generator potentials we applied either H-7 (100μM) or sphingosine (10-30μM) to isolated nervous systems 15 min. or 24 hrs. after the application of light paired with 5-HT (10^{-4}M). Light and 5-HT produced an increase in the peak and plateau phases of generator potentials evoked by a 2 min. light step as previously reported (mean increase from baseline; peak = 15.6%, plateau = 24.2%; $P<.05$). H-7 applied after the 5-HT did not reduce either the peak or plateau phase of the generator potential (mean increase from baseline in the presence of 5-HT and H-7; peak = 16.5%, plateau = 25.1%; $P<.05$). Similar results were obtained after the application of sphingosine (mean increase from baseline in the presence of 5-HT and sphingosine; peak = 15.2%, plateau = 24.7%; $P<.05$). Sphingosine and H-7 did not reverse the long-term enhancement of the plateau phase of generator potentials recorded from lateral B-photoreceptors. These results indicate that the expression of short and long-term enhancement of the generator potentials does not require persistent protein kinase activity.

509.11

EYE REMOVAL EXPERIMENTS DEMONSTRATE THAT THE LIGHT RESPONSES OF IDENTIFIED PEDAL NEURONS IN HERMISSENDA ARE DUE TO SYNAPTIC INPUT FROM PHOTORECEPTORS. T.M. Hodgson and T. Crow, Dept. of Neurobiol. and Anat., Univ. of Texas Med. Sch., Houston, TX 77225.

As a first step in the investigation of the neural circuitry underlying the expression of behavioral changes associated with conditioning in Hermissenda four light-responsive cell types were identified from intracellular recordings of putative motor neurons in the pedal ganglia. Results of experiments using Ca^{2+} blockers and axotomy of the optic nerve showed that the light responses are not intrinsic to the pedal cells and suggested that the pedal cells' light responses are due to synaptic input from the photoreceptors (Hodgson & Crow, 1987). However, extraocular photoreceptors may contribute to the light responses of the identified pedal cells. To determine if the pedal cells receive input from other light responsive neurons whose cell bodies or axons may have been cut or damaged during the axotomy experiments, the light response of each pedal cell type was recorded before and after the eyes were removed by a suction technique that left all other nervous system structures intact. The light responses of the four cell types were abolished after eye removal, confirming the results of previous experiments using synaptic blockers and axotomy.

The anatomy and light responses of the identified pedal cells have also been further characterized. Lucifer yellow cell fills show that all four cell types have one process that exits the nervous system via a identified pedal nerve. Cells P7, P8 and P10 exit in nerve P3; P9 exits in nerve P1. Intracellular recordings of pedal cell responses to 5-10 minute light steps show that P7, which has an inhibitory light response, shows a decrease in spike frequency during the light step ($p<0.05$). P8 showed no significant change in spike frequency during a long light step, however this neuron does exhibit an excitatory "on" response to light.

509.12

IDENTIFICATION OF NEURAL PATHWAYS MEDIATING POSITIVE PHOTOTAXIS IN *APLYSIA*. F.M. Kuenzi, S. Cruikshank*, A.M.B. Storer* and T.J. Carew. Depts. Biol. and Psychol., Yale Univ., New Haven, CT, 06520

Aplysia californica wave their head and anterior body in side to side searching movements when suspended underwater above a substrate. A cellular analysis of this complex behavior would be greatly facilitated by the ability to modulate this response in a rapid, predictable way. We here report that *Aplysia* show a positive phototaxis which reliably modulates headwaving. Moreover, we have identified a set of visual pathways that are necessary for the expression of the phototaxis.

To examine phototaxis, animals (n=14) were suspended in a tank of sea water, and given 5 presentations (1 min each) of a dim directional light at an angle of 70° either to the left or right of their resting long axis (defined as 0°). In the presence of the light animals continued to head wave, but their overall orientation was significantly biased toward the light source ($\bar{x}=27°\pm7.6$, $p<.003$).

We next examined the role of primary visual pathways, the optic and rhinophore nerves, in the phototactic response. Chronic transection of these nerves prior to testing (n=8) significantly reduced phototaxis compared to sham operated controls (n=5, $p<0.005$). The sham controls however, showed normal, significant phototaxis ($p<0.01$).

We have recently developed a preparation for recording the activity of central neurons during head waving. The ability to modulate this behavior with light will be an important tool in analyzing the cellular mechanisms underlying this complex behavioral response.

509.13

INHIBITION IN JUVENILE *APLYSIA*: MASKING OF AN OCCULT FACILITATORY EFFECT OF CONNECTIVE STIMULATION. T.G. Nolen and C. Bittner*. Dept. of Biology, Univ. of Miami, Coral Gables, FL 33124 and the Marine Biological Laboratory, Woods Hole, MA 02543.

Noxious stimulation produces several forms of non-associative learning in *Aplysia californica*. In adults, a shock to the tail produces two *apparently* sequential effects on the siphon withdrawal reflex -- initial reflex inhibition followed by sensitization some 30 minutes later (Marcus et al, '88 SCI 241:210). During ontogeny, sensitization does not emerge until quite late in juvenile development (Rankin & Carew, '88, J. Neurosci 8:197; Nolen & Carew, '88, J. Neurosci 8:212).

The late emergence of sensitization may reflect the delayed maturation of the facilitatory process and/or the inhibition of an emerging, but weak form of sensitization. To test the latter possibility, we investigated the ability of connective stimulation (an analog of tail shock) to produce inhibition and facilitation of the siphon nerve evoked EPSP in R2. We used phosphodiesterase inhibitors (e.g. IBMX) to enhance the facilitatory effects of connective stimulation by elevating the levels of cAMP in early stage 12 juveniles, a time when sensitization is emerging.

Most Early stage 12 preparations showed inhibition, rather than facilitation. Early to Mid stage 12 preparations were assigned to one of two classes, NON-FACILITATED or FACILITATED, depending on the effect of connective stimulation on the evoked, complex EPSP. In NON-FACIL preparations 0.10-0.20mM IBMX enhanced both spontaneous and evoked EPSPs. Significantly, for about 20min following washout of IBMX, connective stimulation caused significant facilitation of the evoked EPSP (mediun relative response = 136%, $p<0.032$). IBMX treatment caused the suppression of facilitation in the FACIL group ($p<0.05$). We conclude that an otherwise occult form of facilitation is present by early stage 12 -- before the behavioral emergence of sensitization -- and that connective stimulation activates a parallel inhibitory pathway that is also enhanced by IBMX.

SUPPORTED BY A UM SUMMER RESEARCH FELLOWSHIP AND AN S.W. KUFFLER MBL SUMMER FELLOWSHIP (TGN).

509.14

DEVELOPMENT OF LONG-TERM MEMORY IN *APLYSIA*: LONG-TERM SENSITIZATION IS PRESENT WHEN SHORT-TERM SENSITIZATION FIRST EMERGES. T.J. Carew, W.G. Wright*, E.F. McCance*. Dept. Psychology, Yale University, New Haven, CT, 06520

A central question in the neurobiology of learning concerns the mechanistic relationship between short- and long-term memory. The siphon withdrawal reflex of *Aplysia* provides a useful system for examining this relationship, since it exhibits both short- and long-term sensitization. The present study addresses the hypothesis that long-term memory requires the activation of short-term memory processes, by examining when these two forms of memory emerge developmentally. As a first step in studying this question, we have examined whether the long-term memory for sensitization is present in the developmental stage in which short-term sensitization is first expressed (Late Stage 12, Rankin and Carew, 1988).

We first examined long-term sensitization in adult animals. Duration of the siphon withdrawal response to water-jet stimuli to the siphon was measured before, and one day after sensitization training (2 sessions of repeated strong tail-shock). Consistent with previous studies. animals showed significant sensitization one day after sensitization training ($\bar{x}=161\%$; $p<.002$; n=32), and were significantly more sensitized than unshocked controls (n=32) ($p<.001$). Using identical procedures we next examined Late Stage 12 juveniles. These animals also showed significant sensitization one day after training ($\bar{x}=180\%$; $p<.006$; n=10), and again this increase was significantly greater than that of unshocked controls (n=10) ($p<.001$).

Our results indicate that long-term memory of sensitization training can be observed during development as soon as the short-term process is in place. It will now be of interest to examine the possibility that long-term sensitization can be expressed even before short-term sensitization emerges.

509.15

ONSET OF STRUCTURAL CHANGES AT IDENTIFIED SENSORY NEURON SYNAPSES AND THE ACQUISITION OF LONG-TERM SENSITIZATION IN APLYSIA. C. H. Bailey and M. Chen. Ctr. for Neurobiol. & Behav., Dept. of Anat. & Cell Biol., Neurol., & Psychiat., Columbia P&S, and NYSPI, NY, NY

We have begun to explore the morphological basis for the graded nature of long-term sensitization by examining the structural events that may accompany its acquisition and initial phases of maintenance. Toward that end, we have quantitated changes in the total number of varicosities of single HRP-labeled sensory neurons examined at different intervals following the completion of training. Long-term sensitization was produced using either a shortened (1.5 hr) protocol that results in a memory lasting several days or a more prolonged (4 day) protocol that results in a memory lasting several weeks (Frost et al., PNAS, 1985). Preliminary results suggest no evidence of synapse formation at 1 hr following the end of training in sensitized (1435 ± 99 S.E.M., N=6) vs. control animals (1569 ± 53, N=2). By contrast, sensitized animals examined at later times 12-16 hrs (1832 ± 38, N=5 vs. 1453 ± 129, N=3, t=4, $p<0.01$) and 24-28 hrs (2052 ± 46, N=8 vs. 1517 ± 19, N=6, t=9.5, $p<0.001$) demonstrated a progressively larger number of varicosities. In addition, sensitized animals examined 24-28 hrs following the 4 day protocol displayed even more pronounced changes (2670 ± 72, N=4 vs. 1310 ± 118, N=4, t=9.9, $p<0.001$). These results indicate that increases in the number of sensory neuron varicositites parallel in a graded fashion the graded emergence of the memory and suggest a role for synapse number changes in the formation of long-term sensitization.

509.16

LONG-TERM CHANGES IN THE MORPHOLOGY OF *APLYSIA* SENSORY NEURONS *IN VITRO* DUE TO 5-HT RESEMBLE DEVELOPMENTAL GROWTH IN BEING REGULATED BY THE POSTSYNAPTIC MOTOR NEURON. D.L. Glanzman, S. Schacher, and E.R. Kandel. Ctr. Neurobiol & Behav. HHMI, Columbia Univ., & NYS Psych. Inst. NY, NY 10032.

The development of *Aplysia* sensory neurons in dissociated cell culture is regulated by the presence of an appropriate target motor neuron (Glanzman et al., *Soc. Neurosci. Abstr.*, 1987). Sensory neurons co-cultured with the identified motor neuron L7 have more complex morphologies than do sensory neurons cultured alone. Moreover, the outgrowth of the sensory neurons during development *in vitro* appears to be guided by the processes of the motor neurons. We have now found that the postsynaptic cell is also required for long-term morphological changes in sensory neurons in response to a modulatory transmitter, 5-HT. Applications of 5-HT produced an increase in the number of varicosities on the neurites of sensory neurons only when co-cultured with L7 cells. Serotonin produced no apparent long-term increase in varicosities when sensory neurons were cultured alone. The increase in the number of sensory neuron varicosities induced by 5-HT was not cell-wide but seemed restricted to neurites which contacted the major processes of L7. Our results suggest that some signal from the postsynaptic motor cell regulates long-term changes in the morphology of sensory neurons, both during development and due to modulatory transmitters important for learning and memory.

509.17

RULES FOR BEHAVIORAL CHOICE IN *APLYSIA FASCIATA*. I. Ziv*, S. Markovich* & A.J. Susswein. Dept Life Sci, Bar-Ilan Univ.,Ramat Gan 52 100, Israel.

Aplysia has been widely used to study neural mechanisms controlling specific behaviors. However, mechanisms underlying integration between different behaviors have not been examined previously.

Rules underlying choice of feeding, reproduction (courtship, male or female mating, egg-laying) or mobility (immobile, move in place, crawling, swimming) were determined, by examining time budgeting, and time of day that behaviors occur, when food and potential mates were present or absent.

1. Animals rarely feed or locomote while engaged in a reproductive behavior. By contrast, different reproductive behaviors often occur simultaneously.

2. A common arousal mechanism controls feeding and mating. However, feedback control of arousal by feeding and mating is asymmetric. Feeding inhibits the arousal, while mating excites it. Thus, in absence of food time spent mating *increases*, while in absence of mates time spent feeding *decreases*.

3. Time distributed between locomotion (crawling and swimming) and inactivity (immobile and moving in place) is unaffected by addition of food or mates, suggesting that the 4 mobility states fill in the time left over after motivational needs to feed and reproduce are satisfied.

4. Behaviors differed in tendency to occur at specific times of day. Swimming had the strongest tendency to clustering, while moving in place had the least.

5. Behaviors differed in the degree that they were modulated by a circadian oscillator. Variation in crawling, moving in place, or egg-laying was never circadian, while immobile, swimming, mating and eating were circadian in at least one condition. Peaks of swimming and immobile occurred out of phase with one another, as did peaks of mating and feeding.

6. Change of behavioral state altered the relative contribution of the circadian component to daily behavioral variation. When food and mates were present, immobile and swimming became less circadian, while presence of mates made feeding become more circadian.

The data indicate that neural circuits governing choice between behaviors are affected by circadian oscillators and by motivational state. Oscillators and motivational variables interact in complex ways.

509.18

THE PEPTIDERGIC MODULATION OF MOTOR OUTPUT DURING EGG-LAYING BEHAVIOR IN THE SNAIL *LYMNAEA STAGNALIS*. R.F. Jansen, G.P. Ferguson and A. ter Maat. Dept. of Biology, Vrije Universiteit, De Boelelaan 1087, 1081 HV Amsterdam, The Netherlands.

Egg-laying in the fresh water snail *Lymnaea stagnalis* is characterized by a stereotyped behavior lasting 2-3 hours. This behavior is an all-or-none event that is triggered by a synchronous burst of spiking activity in the Caudo-Dorsal Cells (CDCs), a group of neuro-endocrine cells in the cerebral ganglia of the central nervous system. The overt egg-laying behavior of *Lymnaea* is comprised of three distinct phases: resting, turning and oviposition, each named after the activity of the animal during these phases.

The typical patterns of motor activity of the head/foot and the buccal mass of the animal that occur during egg-laying behavior are caused by a coordinated action of sensory feedback from the ovotestis, and several peptidergic hormones and putative neurotransmitters that are released from the CDCs during the discharge into the blood and to the CNS, respectively.

We investigated the effects of one of these peptides, Caudo-Dorsal Cell Hormone (CDCH), on the motor output of the CNS. To this end, we have analysed the locomotion of the animal during the different phases of egg-laying behavior using a video-digitizer. The motor-output of the CNS was analysed *in vivo* and *in vitro* using permanently implanted electrodes and a novel waveform recognition program. Our results show that application of CDCH modulates the motor output of the CNS. However, the sensory feedback from the ovotestis is needed to trigger the full complement of the behavior *in vivo*.

509.19

EFFECTS OF CONOPRESSIN G ON APLYSIA SENSORY NEURONS. M. Martinez-Padron & K. Lukowiak. Medical Physiology, University of Calgary, Alberta Canada T2N 4N1.

Conopressin G, a peptide of the vasopressin-oxytocin family has been shown in Aplysia to suppress the amplitude of siphon-evoked gill withdrawal behaviour (GWR). Certain neuromodulators affect the electrical properties of the sensory neurons.

We tested the ability of Conopressin G to affect the duration of the sensory neuron action potential (AP) during frequency dependent spike broadening (FDSP). Superfusion of Conopressin G (1 μM) over the isolated parietal visceral ganglion (PVG) increased the duration of the sensory neuron AP in 60% of the cases. Interestingly, the duration of pleural sensory neurons APs was unaffected by Conopressin G. Conopressin G also significantly reduced AP accommodation in both abdominal and pleural sensory neurons. Conopressin G (1 μM) applied to the bath greatly increased the number of APs elicited in response to long (500 ms) depolarizing current injection. This effect is specific to Conopressin G and does not occur in response to the same concentration of either Conopressin S or arginine vasotocin. The physiological significance of these effects are unclear in view of the suppressive action of Conopressin G on GWR. However, the results indicate that changes at specific loci within the central nervous system must be carefully evaluated as neuronal correlates of behavioral plasticity.

509.20

OPTICAL RECORDING OF NEURONAL ACTIVITY: ABSORPTION SIGNALS FROM NEURONS INJECTED WITH VOLTAGE SENSITIVE DYES. D. Zecevic and C. Xiao. Univ.Belgrade, Yugoslavia; Yale Univ.Sch.Med. New Haven, CT 06510.

We investigated optical signals from individual neurons from Helix selectively stained by intracellular dye injection. Our aim is to find signals that are large enough to allow the analyses of regional properties of individual neurons in the intact ganglia. We concentrate on absorption mode of measurements for two main reasons: 1) in terms of photodynamic damage fluorescent dyes are significantly worse than absorption dyes; 2) intracellular dye application results in high background fluorescence which reduces signal-to-noise ratio.

Negatively charged oxonol dye RGA-509 that gave largest absorption signals after extracellular application did not produce any signal when injected into the neurons. Both fluorescence and absorption signals were obtained using positively charged styryl dyes RH-461 and RH-437; signal-to-noise ratio was 6 fold smaller in absorption. We are currently screening analogs of merocyanine-rhodanine and merocyanine-oxozolone type for larger absorption signals.

INVERTEBRATE SENSORY SYSTEMS I

510.1

DEVELOPMENT AND EVOLUTION OF SEGMENTALLY HOMOLOGOUS SENSORY SYSTEMS IN THE LOCUST. H. Reichert and T. Meier*. Department of Zoology, University of Geneva, CH-1211 Geneva, Switzerland.

Our goal is to understand general organizational principles of metameric nervous systems. For this we are studying the differentiation of a homonymously segmented embryonic nervous system into a segmentally specialized adult nervous system. During the development of the thoracic and abdominal segments in the locust identified clusters of mechanosensory neurons derive at characteristic positions along the body wall ectoderm. In a lateral cluster all of these sensory neurons differentiate as multicellular aggregates by epithelial invagination, cell migration and subsequent projection onto the intersegmental nerve. They give rise to the wing chordotonal organs in the thoracic segments, to the auditory organ in the first abdominal segment and to the pleural chordotonal organs in the remaining abdominal segments. In a dorsal cluster an identified neuron differentiates into the wing stretch receptor in the pterothoracic segments and becomes involved in controlling the ventilatory rhythm in the abdominal segments. Thus a number of adult sensory structures which are involved in completely different behavioral tasks are segmentally homologous and probably share a common evolutionary origin. Supported by the SNSF.

510.2

EMBRYONIC DEVELOPMENT OF LOCAL SPIKING NEURONS IN THE LOCUST. D.Shepherd* and G.J.Laurent* (SPON:G.A.Lnenicka).Dept. of Zoology University of Cambridge.Cambridge CB2 3EJ England.

We are examining the embryonic development of 2 groups of identified local interneurons in the locust. These neurons integrate sensory input from the legs and map this input as a set of overlapping receptive fields. The aim of this work is to identify the parent neuroblasts (NBs) of these interneurons and understand the mechanisms that control the initial formation of their receptive field properties.

Using intracellular injection of cobalt we have described the morphological development of the interneurons. This starts from the earliest stage at which they can be identified (60%), through to the first days of larval life. The development of the presynaptic leg exteroceptors coincides with that of the interneurons.

Two NBs have been identified whose progeny form at least part of the complement of interneurons.

Supported by SERC and a Royal Society Locke Fellowship to G.J.L.

510.3

ELEMENTS OF THE ANCESTRAL FLIGHT MECHANISM IN SECONDARILY FLIGHTLESS GRASSHOPPERS: HOMOLOGUES OF THE "WING-HINGE STRETCH RECEPTORS". EA Arbas ARL Div. of Neurobiology, Univ. of Arizona, Tucson AZ 85721.

All locusts and grasshoppers have evolved from ancestors capable of flight, yet many have become secondarily flightless. We have shown previously, by comparing the flightless grasshoppers Barytettix psolus with other forms able to fly, that adult B. psolus lack hindwings, have vestigial forewings incapable of active movement and have eliminated thoracic muscles that are specialized for flight in locusts. Yet, many elements of the ancestral flight mechanism can be identified, e.g. pterothoracic dorsal longitudinal (DL) "flight" muscles differentiate and are innervated during development, then atrophy or degenerate by adulthood, while small DL motorneurons persist. Sensory neurons homologous to the stretch receptors (SRs) of the locust wing-hinge occupy the hinge-less cuticle in B. psolus. SRs in locusts have been shown by others to monitor wing elevation, and to function as elements of the flight pattern generator. We wish to understand how the structure and function of the SRs have been modified in the flightless form. Detailed quantitative comparisons of SR projection patterns in thoracic CNS revealed by cobalt backfilling in B. psolus and grasshoppers able to fly, Schistocerca americana and Melanoplus differentialis show that although basic projection pathways are essentially conserved across the three genera, certain identifiable higher-order branches are absent while others are reduced in B. psolus, leaving specific regions of neuropil uninnervated. These anatomical differences may underlie changes in synaptic connectivity and function, relative to an ancestral role in flight. Understanding their basis may help clarify how neural circuits are modified as behavior evolves. [Supported by USPHS grant NS 07309 and a grant from the Whitehall Foundation.]

510.4

AFFERENT PROJECTIONS OF THE LEG CAMPANIFORM SENSE ORGANS IN THE FLY PHORMIA. D.J. Merritt* and R.K. Murphey. Neurobiology Research Center, SUNY Albany, NY 12222.

The hair-like sensilla on the legs of the blowfly, Phormia can be assigned to one of three categories based on the neuropil region to which their axons project: gustatory, tactile and proprioceptive (Pollack et al., Abstr. Soc. Neurosci., 14, 869, 1988). Here we examine the dome-shaped campaniform sensilla found singly or in clusters near the joints on the trochanter, femur, tibia and tarsi. They are mechanoreceptors stimulated by stresses in the cuticle and are believed to function as proprioceptors providing feedback on leg movement or contact with the substrate.

The campaniform sensilla display two types of projection patterns in the CNS. One class projects to the intermediate region of the leg neuropil, sharing this pattern with the hair plate sensilla which are clustered near the joints and also function as proprioceptors. Another class projects into a region of neuropil dorsal to the commissure of the paired leg neuromeres which is not normally associated with leg afferents. Some also project to this same region in adjacent neuromeres. Thus the projection-based categorization of sensilla encompasses one class of campaniform sensilla in the proprioceptive category. It must be extended to include the second class of campaniform sensilla whose projection patterns are currently being examined in detail. Supported by NSF grant BNS8719377 and NIH grant NS15571 to RKM.

510.5

PLURISEGMENTAL INTERNEURONS CARRYING ANTENNAL -DERIVED TACTILE SENSORY INFORMATION IN THE COCKROACH. J.A. Burdohan* and C.M. Comer (SPON: R. Ruth). Dept. Biological Sciences, Univ. of Illinois, Chicago, IL 60680.

The wind-sensory cercal-to-GI pathway of the cockroach, Periplaneta americana, is known to be involved in escape behavior. Recent work in our lab has demonstrated that antennal mechanosensory pathways also may mediate evasive responses to certain types of predators. To determine how these evasive responses are controlled, we have recorded from and labelled cells that carry antennal-derived tactile information from the head ganglia to thoracic motor centers.

Axons were impaled in the neck connectives, and after physiological study a cell was injected with cobalt hexamine chloride to allow subsequent anatomical reconstruction. Cross sections of the neck connectives showed that there are as many as 15 large diameter fibers at this level. We sampled these and other cells at the cervical level in order to determine which responded to tactile stimulation of the antennae. We found several classes of cells that carried descending tactile information. Two were defined on the basis of the location of the cell body [either in the supraesophageal or the subesophageal ganglion]. In addition, there were three subclasses based on whether an individual cell responded to the contralateral, ipsilateral, or both antennae.

We are now examining the motor responses generated by stimulating each of these cells, and relating this to the leg movements during a tactually generated evasive response.

(Supported by NSF grant # BNS-8617393 to C.M.C.)

510.6

CONNECTIONS BETWEEN VENTRAL GIANT INTERNEURONS AND THORACIC INTERNEURONS OF THE COCKROACH OCCUR SPECIFICALLY ON THE VENTRAL MEDIAN BRANCH OF THE TIS. J.L. Casagrand and R.E. Ritzmann. Dept. of Biology, Case Western Reserve Univ., Cleveland, OH 44106.

The ventral giant interneurons (vGIs) of the cockroach, Periplaneta americana, connect to a large population of thoracic interneurons (type A TIs) which appear to play an important role in orienting the cockroach during wind-mediated escape. All type A interneurons are characterized by a prominent branch located in the ventral median region of the thoracic ganglion in which their soma resides. For any TI the presence of this ventral median (VM) branch on one or both sides of the ganglion can be used to predict connectivity with specific vGIs, suggesting that this is indeed the locus of synaptic connection.

We have employed various morphological and developmental techniques to verify that VM branches are indeed the sites of connection between vGIs and type A thoracic interneurons. We examined overlap directly at the light microscopic level by filling GIs and TIs with differently colored fluorescent dyes (Ethidium Bromide, Lucifer Yellow) in the same preparation. Overlap was observed between vGIs and the VM branches of TIs. In addition experiments were performed in which half the GI input to TIs was surgically removed early in development. When these animals reached adulthood, type A TIs were observed and compared to normal animals. TIs that normally have bilaterally symmetric VM branches were now markedly asymmetric. The VM branch on the deprived side was consistently shorter than that on the control side. Another pair of prominent branches called lateral branches were unaffected by this surgery. These branches are thought to receive inputs from sensory structures on the legs. We are presently attempting to confirm these results at the EM level.

Supported by NRSA Training Grant HD 07104 to J.L.C. and NIH grant NS 17411 to R.E.R.

510.7

ANATOMY AND PHYSIOLOGY OF IDENTIFIED CERCAL AFFERENTS IN THE COCKROACH. Darryl L. Daley. Department of Physiology, National College of Chiropractic, Lombard, Il 60148.

The wind sensitive afferents (**WSA's**) of the cercal-to-giant interneuron system of the American cockroach encode the information necessary for the animal's well known escape response to wind puffs. In this study the directional response characteristics of **WSA's** were correlated with the afferents central anatomy in the terminal abdominal ganglion. The anatomy and physiology of cercal afferents was studied via intracellular recording and staining. Wind puffs of varying directions were delivered while recording intracellularly from **WSA's**. All **WSA's** studied showed ongoing activity and responded to wind puffs over approximately 180° of wind angle as previously shown (Westin, 1979). The identity of **WSA's** was established by intracellular injection of cobaltic hexamine chloride and subsequent wholemount silver intensification. The terminal aborizations of the **WSA's** project to a well defined region of the terminal ganglion (a region where the giant interneurons have numerous dendritic processes).

510.8

ION CHANNELS IN ISOLATED MECHANOSENSORY NEURONS FROM THE CONNECTIVE CHORDOTONAL ORGAN IN THE PEDICEL OF THE AMERICAN COCKROACH. L.L. Stockbridge* and A.S. French. Dept. of Physiology, Univ. of Alberta, Edmonton, CANADA.

Single mechanosensory neurons were isolated from the connective chordotonal organ of adult Periplaneta americana antennae using a combination of mechanical and enzymatic dissociation. Freshly dissociated cells were examined by light microscopy and by transmission and scanning electron microscopy. These studies verified that the cells were mechanoreceptors and that the cell body, the distal dendrite and part of the sensory cilium (the mechanosensory ending) survived the dissociation procedure. Often the cell body retained part of its glial wrappings, but distal structures were free of any intact glia. The patch clamp technique was employed to identify ion channels involved in the transduction of mechanical stimuli in these cells. Preliminary experiments have revealed the presence of a stretch-activated channel with a conductance of approximately 100 pS which is permeant to both potassium and sodium ions. At least two other potassium channels are presently being identified.

Supported by Canadian M.R.C. and A.H.F.M.R.

510.9

THE ROLE OF IDENTIFIED WIND SENSITIVE LOCAL INTERNEURONS IN THE CRICKET CERCAL SENSORY SYSTEM. Deana A. Bodnar, Shai N. Gozani*, Rocky Nevin*, and John P. Miller. Graduate Group in Neurobiology, University of California, Berkeley, CA 94720.

Experiments were performed to assess the role of a group of identified local interneurons (INs) in the processing of wind information by the cricket cercal sensory system. The stimulus-response characteristics of giant ascending INs were measured before and after photoinactivation of a subset of the local INs. The activity of all the giant INs was monitored simultaneously using multiple extracellular electrodes and multi-unit spike discrimination software. The results indicated that the local INs have a functional excitatory influence upon giant INs with similar directional sensitivities.

In addition, simultaneous intracellular recordings of a local IN and a giant IN with similar directional sensitivities were obtained. Depolarization of the local IN caused an increased excitability in the giant IN. Computer reconstructions of these pairs of cells showed a high degree of dendritic overlap. Therefore it is possible that the observed excitatory influences are mediated by direct synaptic contact. Supported by NSF and NIH grants to JPM.

510.10

USING COMPLEX INPUT IMPEDANCE MEASUREMENTS, LASER PHOTOINACTIVATION AND COMPUTER MODELING TO DETERMINE STRUCTURE-FUNCTION RELATIONSHIPS IN CRICKET INTERNEURONS. H.M.Brew*, J.W.Tromp*, R.H.W.Nevin* & J.P.Miller. Dept. of Zoology, UC Berkeley.

Directional sensitivities of wind-sensitive interneurons (INs) in the cricket cercal system depend on aspects of their complex dendritic structure. To gain a more quantitative understanding of synaptic integration in these INs, we have developed compartmental computer models. The anatomical parameters for the models were measured from stained INs using computer reconstruction software developed in this lab. The electrical parameters were derived from experiments in which the complex input impedances of INs were measured using a switched single-electrode current clamp. In some experiments, a laser was used to photoinactivate specific sites in the cell's branching structure, and the impedance measurements were repeated. Such experiments allow determination of the distribution of membrane conductances in the model. The modeling calculations indicate that 1) different parts of a single arbor are relatively isopotential in response to synaptic input, but 2) the different arbors are isolated from one another (and from the SIZ) by significant complex impedances. These impedances strongly influence the relative weighting of synaptic inputs from sensory afferents of different directional selectivities. Supported by SERC/NATO (HB) & NSF (JT, RN & JM).

510.11

CHARACTERIZATION OF SYNAPTIC INTEGRATION BY ELECTROPHYSIOLOGICAL ANALYSIS AND COMPARTMENTAL MODELING J. Dye, J. Tromp* & R. Nevin* Dept. of Zoology, Univ. of Calif., Berkeley, CA 94720.

Understanding the information processing of neurons in functional systems requires knowledge both of the synaptic and active membrane properties, and of the interaction of these active regions across the structure of the cell. An approach to the problem of analyzing remote synapses was suggested by Carnevale & Johnston (J Neurophysiol 47:606 1982). Their two-port analysis neglects anatomical considerations and describes steady-state synaptic attenuation by purely electrophysiological means.

We are using this approach in conjunction with a precise compartmental model to study synaptic integration in an identified interneuron of the cricket cercal sensory system. Electrophysiological measures fix free parameters of the model, and the model in turn indicates the degree to which some of the approximations of the two-port analysis are correct. Thus the model constrains an interpretion of the physiological results by integrating various data into the true anatomical structure, and extends the power of the two-port analysis to nonideal conditions. (This work supported by an NSF grant to J.P. Miller, and NIH to JD)

510.12

IPSP-EXCITATORY INTERACTIONS IN CRAYFISH STRETCHRECEPTOR. W. Buño*, J.C. Barrio* and A. Araque*. (SPON. M.A. Morán). Instituto Cajal, Velázquez 144, Madrid, Spain.

Regular IPSP or brief hyperpolarizing current pulse barrage effects on coding of injected excitatory sine currents were examined in the slowly adapting receptor (RM1). The postsynaptic contribution was determined by comparing: (a) IPSP and pulse peak hyper- (H) and subsequent threshold depolarizations (T), and HT slopes; (b) times between an IPSP or pulse and the closest spike preceding it, or following, known as phase (ϕ) and cophase (θ), respectively. Lockings, including S pulses or IPSPs and R spikes and driving at S/R times barrage rate, were ever present. Jumps between S/R ratios occurred at certain excitation values. Lockings showed: (a) gradual ϕ increase, θ decrease at sine de- hyperpolarizing segments, respectively; (b) θ and ϕ paralleled control intervals and inhibitory minus control differences, respectively; (c) T were invariant, H and HT increased and decreased at de- hyperpolarizing segments, respectively, and increased with barrage rate. IPSP and pulse effects were determined by H and HT, and were similar, therefore they were due to postsynaptic properties. The functional meaning can only be conjectured about, but could provide greater control flexibility than the usually proposed summation.

* Supported by DGICT grant to W.B.

510.13

ROLE OF SENSORY NEURONS IN BEHAVIORAL CHANGES DISPLAYED BY DEVELOPING CRAYFISH. E.M. Leise, R.A. Fricke and D.H. Edwards, Biol. Dept., Georgia State University, Atlanta, GA, 30303.

Stimulation of abdominal tactile afferents excite the LG command neurons for escape tail-flip by two pathways: a monosynaptic electrical connection and a disynaptic pathway with electrical and depression-prone chemical synapses. During development, when the animals are 3 to 4 cm long, the disynaptic pathway replaces the monosynaptic connection as the functional means for exciting LG. At this time, LG tailflip begins to display habituation to repetitive stimuli. We are studying behaviorally correlated changes in the synaptic interaction between sensory neurons and LG during development. In adults, we have found that electrical synapses onto LG from primary afferents and sensory interneurons (SIs) rectify and can be inhibited by postsynaptic depolarization. The SIs are normally depolarized with respect to LG at rest, which forward-biases the synapses. In juveniles, which have fewer primary afferents, we found that dendritic branching patterns of LG and the SIs are simpler and less extensive than in adults. Supported by NIH Research Grant NS26457 to DHE.

510.14

MECHANORECEPTION IN THE FIRST ANTENNA OF MARINE COPEPODS. P.H. Lenz[1]*, J. Yen[2]* and D.K. Hartline[1], [1]Bekesy Lab, Univ. Hawaii, Honolulu, HI 96822; [2]Marine Sciences Res. Ctr., SUNY, Stony Brook, NY 11794.

We examined the properties of mechanosensory neurons in the first antennae of 10 species of pelagic calanoid copepods. Neural activity was recorded extracellularly at the base of the first antenna. Controlled mechanical stimuli were delivered with a vibrator driven by a waveform generator. The receptors responded to stimuli between 40 and over 1000 Hz. Unlike the previously described decapod mechanosensors, we found that sensitivity increased with frequency, and at 1000 Hz threshold sensitivities were near 10 nm. Phase-locking of spikes to oscillatory stimuli was observed at frequencies up to 500 Hz.

Different species showed different types of neural responses. Responses in most were characterized by a large number of small spikes, where individual units could not be identified from extracellular recordings. In contrast, the two bay species, Labidocera madurae and Acartia fossae, had a small number of large amplitude units, and individual units responded when a single hair was stimulated.

Morphological studies with scanning electron microscopy indicated that hairs are structurally constrained to move in specific planes, and both simple smooth and feathered hairs occur. All species studied had exceptionally long first antennae in relation to body length with longer hairs on the distal segments. This structural organization may optimize mechanosensory capabilities.

Supported by ONR grant N00014-87-K-0181 and a UH Research Council award to JY.

510.15

EVIDENCE FOR HISTAMINE AS A PUTATIVE INHIBITORY NEUROTRANSMITTER IN THE OLFACTORY LOBE OF THE SPINY LOBSTER. E. Orona, B-A. Battelle, and B. W. Ache (SPON: S. Edwards). The Whitney Lab and Depts. Zoology & Neuroscience, Univ. Florida, St. Augustine, FL 32086.

The biogenic amine, histamine (HA), has been implicated to act in the olfactory CNS of arthropods (Maxwell et al., Comp. Biochem. Physiol., 61C:109, 1978) and mammals (Rhoades et al., Neurosci. Abst., #474.2, 1988). We now report that olfactory lobe (OL) tissue of the spiny lobster is capable of synthesizing HA from its radioactive precursor, histidine. Specific HA-like immunoreactivity (polyclonal antibody, Chemicon) occurs in: (1) cell body clusters of olfactory interneurons adjacent to the OL, (2) the outer cap region of the OL glomeruli, and (3) small cells, presumably glia, adjacent to the glomeruli that are possibly involved with HA uptake or inactivation. The distribution of HA in the OL glomeruli combined with recent evidence from our lab for a HA-gated Cl^- channel on lobster olfactory receptor cells (McClintock et al., submitted) suggests that HA functions as an inhibitory neurotransmitter in the OL. Combined pharmacological and physiological experiments to test this hypothesis are in progress.

(Supported by NSF Awards BNS 86-07660 and 88-10261.)

510.16

NEURONAL ORGANIZATION AND FUNCTION OF THE PECTINAL SENSORY SYSTEM IN SCORPIONS. P. H. Brownell. Deptartment of Zoology. Oregon State University, Corvallis, Oregon 97331.

The pectines are large, sexually dimorphic appendages located on the mid-ventral surface of all scorpions, primitive (Silurian, aquatic) and modern (terrestrial). Behavioral observations of Paruroctonus mesaensis indicate the pectines are contact chemoreceptors of general sensitivity and probably function in male scorpions as pheromone detectors. The chemoreceptive sensilla of the pectines are arranged in discrete patches that together form an organized, two-dimensional array of approximately 100,000 sensilla (males of Parabuthus pallidus), each innervated by approximately 10 sensory neurons. Electrophysiological recordings from single sensilla on the pectines show each contains one mechanoreceptor and several chemoreceptors that respond to aqueous and nonpolar solutions applied to the sensillum tip. Cobalt infusion and intensification of chemoreceptor axons revealed a central projection that is topographically organized and sexually dimorphic in 5 species (3 of 6 extant scorpion families). This organization is confirmed by degeneration studies showing topographic ordering of synaptic terminals in glomerular and layered neuropil. Based on these findings and other behavioral and biochemical studies, we hypothesize that the primary function of the pectinal sensory system is to guide orientation to chemical signals borne on the substrate.

Supported by NSF-BNS 8709890

510.17

ULTRAMICROSCOPIC EXAMINATION OF THE RETINA OF THE WOLF SPIDER LYCOSA PUNCTULATA. R. S. Lizotte(SPON: L. Luckenbill). Dept. of Zoological and Biomedical Sciences. Ohio University, Athens, Ohio 45701.

Using electronmicroscopy, an ultrastructural examination of the retina of the various eyes of Lycosa punctulata was performed. A layer of pigment cells was found around the rhabdoms of the posterior lateral eyes, posterior medial eyes, and the anterior lateral eyes, but not around the rhabdoms of the anterior medial eyes (AME). The same condition exists for the family Salticidae. Each rhabdom has two rhabdomere faces associated with it, including the rhabdoms of the AME. This condition differs from that described for the Salticidae in which each rhabdom of the AME has five rhabdomere faces associated with it. The rhabdomere faces are made up of microvilli like structures produced by evaginations of the cell membrane. Along the inner faces of the rhabdomeres, pinocytotic vesicles are apparent, lending evidence that Lycosids also exhibit receptor membrane breakdown as has been described for two other families of spiders, the Dinopids and the Salticids. The microvilli of the rhabdomeres show various orientations with regard to the path of incoming light, a condition which may be the basis for detection of polarized light.

510.18

NEPHRIDIAL NERVE CELLS OF THE LEECH ARE SENSITIVE TO EXTERNAL CL^- CONCENTRATION. R.L. Calabrese and A. Wenning . Dept. of Biology, Emory Univ., Atlanta, GA 30322 and Fakultat fur Biologie, Universitat Konstanz, Konstanz D-7750, FRG.

Nephridial nerve cells (NNCs), putative sensory neurons in the leech, Hirudo medicinalis, have a peripheral soma and extensive dendrites that invade a nephridium (Wenning, A., and Cahill, M.A., Cell Tissue Res., 245:397, 1986). Leech blood is normally low in Cl^- (organic acids provide anionic balance), but after feeding Cl^- increases more than two-fold, while cation concentration (mainly Na^+) increases only slightly (Wenning, A., et al., J. Comp. Physiol., 139:97, 1980). NNCs respond to changes in the external Cl^- concentration of the blood and fluids surrounding the nephridium but not to changes in osmolality or Na^+ concentration (Wenning, A., J. Exp. Biol., in press).

Using intracellular recording and switching single-electrode voltage clamp techniques on isolated NNCs with intact dendrites, we showed that low Cl^- (37.6 mM) salines corresponding to normal blood Cl^- levels depolarize NCCs (Vm = -36.7 $\pm$ 9.9 mV), while high Cl^- (132.6 mM) salines corresponding to postfeeding blood Cl^- levels hyperpolarize NNCs (Vm = -55.6 $\pm$ 8.0 mV). NNCs respond to changes in external Cl^- when it is replaced by SO_4^{--}, isethionate, malate, or succinate, but not when it is replaced by Br^-. Low Cl^- salines cause a reversible conductance increase and inward current in NNCs. This inward current is not blocked in 0 mM Na^+ saline or by 5 mM Co^{++} and is not voltage sensitive over the range of -65 to -40 mV.

510.19

EXPOSURE TO 60-HZ MAGNETIC FIELDS INCREASES MORTALITY IN THE LAND SNAIL Cepaea nemoralis: EFFECTS OF DAY-NIGHT RHYTHMS AND LENGTH OF EXPOSURE. S. Lipa*, M. Kavaliers and K.-P. Ossenkopp (SPON: R.R. Shivers). Dept. Psychology and Div. Oral Biology, Univ. Western Ontario, London, Ontario, Canada N6A 5C2.

There is accumulating evidence that powerline frequency magnetic fields can affect a variety of physiological processes. The present experiment examined the effects of various durations (0.5, 2, 12, 48 or 120 h) of day- and night-time exposures to a 1.0 gauss (rms) 60-Hz magnetic field on post-exposure mortality in land snails (Cepaea nemoralis). These snails were injected with morphine or saline and tested for reaction to an aversive thermal stimulus as part of another study. Mortality levels were monitored over a 2 week period and were shown not to be differentially affected by the drug injection procedure. Mortality levels increased significantly ($p<.01$) with increasing length of magnetic field exposure and night-time exposure resulted in greater mortality than day-time exposure ($p<.05$). These results indicate that day-night rhythms are important in determining the magnitude of the magnetic field exposure effect on mortality in these snails. (Supported by NSERC).

511.1

NEURAL BASIS OF HEAD MOVEMENTS EVOKED BY OPTOMOTOR STIMULI IN THE MOTH MANDUCA SEXTA.
Jürgen J. Milde. Zoologisches Institut der Universität, Weyertal 119, D-5000 Cologne 41, F.R.G.

Optomotor stimuli evoke head movements in flying as well as in non-flying animals. Even in an intracellular recording situation the visually guided activity of neck motoneurons and muscles can be monitored. This enables a direct correlation between the responses of visual interneurons in the brain and the motor output.

Intracellular recordings and stainings reveal two major groups of interneurons responding to optomotor stimuli in the brain:

a) non-direction-selective Medulla Giant neurons

b) direction-selective neurons in the Lobula Complex.

Both have a large visual field and connect the optic lobes to the posterior midbrain and the subesophageal ganglion. The majority of the direction-selective neurons prefers a single (horizontal or vertical oriented) direction of stimulus movement. However, neurons with more than one preferred direction in an oblique orientation can occur.

Recordings from direction-selective neck motorneurons supplying two cervical nerves demonstrate that the preferred directions are organized in the same fashion (horizontal or vertical) as found for the visual interneurons. A similar orthogonal organization of the neck motor system has been described recently in flies (Milde JJ, Seyan HS, Strausfeld NJ, J Comp Physiol 160:225, 1987).

511.2

SUBUNIT ORGANIZATION IN ORDINARY GLOMERULI OF MANDUCA AND SARCOPHAGA OLFACTORY CENTERS REFLECTS ORGANIZATION WITHIN SINGLE GLOMERULI OF ORTHOPTERA AND HYMENOPTERA. Alberta S. Kong and Nicholas J. Strausfeld (SPON: J. Angevine) ARL Div. Neurobiology, Univ. of Arizona, Tucson, AZ 85721.

Selective staining of receptors and interneurons reveals specific differences of organization within olfactory neuropils of different insects. In *Calliphora* and *Manduca* a sex-unspecific antennal glomerulus receives more than one morphologically distinct type of receptor terminal and is supplied by several receptor axon bundles each associated with discrete domains in a glomerulus. These are reflected by the lateral extent and stratification of several uniquely identifiable projection neurons (PNs) accompanied by many types of local interneurons [Kanzaki R, Arbas E, Strausfeld NJ, and Hildebrand JG (1989) J. Comp Physiol A. in press]. In *Periplaneta* and the honeybee *Apis*, only one class of receptor terminal innervates any glomerulus. PN dendrites fill a glomerulus and discrete partitions within its neuropil are not resolved. In *Periplaneta* and *Apis* glomerular output neurons (PNs) send axons to complex higher centers (mushroom bodies), which comprise many thousands of interneurons contributing to a sophisticated and highly structured neuroarchitecture [Mobbs, P (1982) Phil Trans Roy Soc Lond 298:309-351]. By comparison, complex glomeruli in Diptera and Lepidoptera are associated with simple mushroom bodies, consisting of a few hundred neurons contributing to a diffuse neuroarchitecture. The anatomy suggests two possible evolutionary lines in olfactory pathway organization. In one, cross-fiber interactions amongst afferent relays are elaborate in the antennal lobes, whereas in Hymenoptera and Orthoptera such interactions may predominate in the mushroom bodies.

511.3

SYNAPTIC ORGANIZATION OF THE MACROGLOMERULAR COMPLEX IN THE ANTENNAL LOBE OF THE MALE MOTH *MANDUCA SEXTA*. F. Yokohari*, L.P. Tolbert, and J.G. Hildebrand (SPON: R. Kanzaki). ARL Div. of Neurobiology, Univ. of Arizona, Tucson, AZ 85721.

The antennal lobe of the brain of adult male *Manduca sexta* comprises a male-specific macroglomerular complex (MGC) and an array of about 60 spheroidal, "ordinary" glomeruli that surrounds a central core of coarse neuropil. The MGC is the principal or exclusive site for primary synaptic processing of sensory information about the female moth's sex-pheromone blend (Matsumoto & Hildebrand, 1981). The MGC has a complicated architecture (Camazine & Hildebrand, unpublished observations; Strausfeld, 1989). The neuronal interactions in the MGC are complex, involving both excitatory and inhibitory synapses (Christensen & Hildebrand, 1987) and a variety of putative neurotransmitters (Homberg et al., 1989).

We are currently analyzing the ultrastructure of the MGC. In reconstructions from serial sections, we identify two main parts, a lobulated "cap" and a deeper "toroid", as well as smaller structures; both the toroid and the cap are 500-600 μm in diameter and >100 μm thick. Presynaptic profiles in the MGC "cap" tentatively fall into five types, containing: (1) medium-sized (ca. 40 nm) round clear vesicles, (2) medium-sized pleiomorphic clear vesicles, (3) large (ca. 90 nm) round clear vesicles, (4) tiny (ca. 30 nm) round clear vesicles, and (5) large round dense-cored vesicles. In work in progress, we are examining the distribution of these synaptic profiles within the subunits of the MGC and identifying the neurons that form each type. [Supported by NIH grants NS20040 and AI23253 and by a fellowship from 福岡大学 , Japan.]

511.4

TWO GROUPS OF ODORANT BINDING PROTEINS IN INSECTS SUGGESTS SPECIFIC AND GENERAL OLFACTORY PATHWAYS. R.G.Vogt and M.R.Lemer*. Section of Molecular Neurobiology, Yale Univ. Sch. of Med., New Haven, CT 06510.

Moths detect pheromone molecules and more general odorants via sensory neurons arrayed along their antennae. The sensory dendrites are encased within a cuticular sheath, and are bathed in an extracellular fluid within the sheath. This fluid has been shown to contain pheromone binding proteins (PBPs) and pheromone degrading enzymes which are uniquely expressed in antennal tissue. We have purified and partially sequenced 7 PBPs from 6 moth species, have found them to be homologous based on sequence identity, but have also found them to be quite variable. The PBPs are recognizable as abundant small proteins (ca. 140 amino acids) which are expressed only or predominantly in male antennae. Comparing the sequences of the first 30 N-terminal amino acids of the 7 PBPs shows that they range in absolute identity from 0.72 to 0.30. However all possess the conserved motif of Val·Met·Lys, Phe·Gly·Lys and Glu at positions 4, 12 and 22 respectively.

In contrast, we have partially sequenced a second group of proteins which are antennal specific but which are expressed equivalently in male and female antennae. Sequences from 4 species show that this group also possesses the sequence motif seen PBPs, and are thus homologous with the PBPs. However, while these sex indifferent proteins are only ca. 0.30 identical to the PBPs, they are 0.95-1.00 identical to each other. These findings suggest that (1) these sex indifferent proteins are general odorant binding proteins (OBPs), (2) that the difference in degree of interspecies variation between PBP and OBP suggests that PBPs evolved from the more highly conserved OBPs, (3) that the variation in PBPs is due to the uniqueness of their respective ligands whereas the OBPs are tuned to a broad range of odorants, and (4) that the pathway for pheromone perception evolved from and became more specialize than the pathway for general odorant perception.

511.5

CENTRAL PROJECTIONS OF CEPHALIC SENSORY NEURONS IN THE LARVAE OF *HELIOTHIS* MOTHS. P.W. Randolph* and H. Itagaki (SPON: J. Hildebrand). ARL Div. of Neurobiology, Univ. of Arizona, Tucson, AZ 85721.

As part of our ongoing work on sensory processing in the insect CNS, we are investigating the central projections of sensory neurons in lepidopterous larvae. Kent and Hildebrand [*Phil Trans R Soc Lond* B 315:1-36 (1987)] described the central projections of sensory neurons in cephalic appendages of the larval sphinx moth *Manduca sexta*. Here, in a complementary study, we report findings from larvae of the pest noctuid moths *Heliothis zea* and *H. virescens*. We cut the sensory appendages and stained their afferent axons with cobalt-lysine followed by silver-intensification, using standard techniques. The tissue was either viewed in whole-mount or embedded in EPON and sectioned.

The central projections of the sensory neurons of the cephalic appendages are very similar in the two *Heliothis* species. Several different neuropil regions in the brain and the subesophageal ganglion receive primary-afferent inputs. The larval antennal center (LAC) in the deutocerebrum and the medial neuropil of the subesophageal ganglion (SEG) receive inputs from both the antenna and the maxilla. The tritocerebrum appears to receive mechanosensory input from tactile hairs on the antenna, labrum, and head cuticle, while sensory afferents from the stemmata (larval eyes) project solely to the optic neuropil in the brain. The stained preparations show that there is an overlap of projections from the antenna and the maxilla, suggesting that the chemosensory inputs from these two appendages are processed in the same neuropil regions in the LAC and SEG.

Our findings from *Heliothis* are almost identical to those from *Manduca*. That the organization of primary sensory pathways is very similar in larvae of two different moth families supports our use of *Manduca* as a model for studies of sensory processing in lepidopterous larvae. [Supported by USDA grant 87-CRCR-1-2362, NIH Postdoctoral Fellowship NS07990, and Monsanto Co.]

511.6

RECTIFIED HIGH-PASS FILTERING IN EARLY MOTION DETECTION.
N. Franceschini, J.M. Pichon, C. Blanes. Neuroinformation Study Group C.N.R.S., Neurobiologie; 31, Ch. J. Aiguier; 13009, Marseilles, France (SPON: ENA)

Directionally selective (DS) motion sensitive neurons carrry out the non trivial computation of *sequence-discrimination*. We have recorded from H1, one of the identifiable, large field DS neurons of the fly *lobula plate* (rev. Hausen K. and Egelhaaf M., in "Facets of Vision" Eds STAVENGA D. G., Hardie R.C., Springer, New York, 1989) upon precise stimulation of a single Elementary Motion Detector (E.M.D.). Two 1µm spots were presented to two neighboring photoreceptor cells in sequence, thereby "simulating" a micromouvement in the visual field . H1 responds with a transient but vigorous spike discharge, provided the sequence simulates motion in the preferred direction (Riehle A. and Franceschini N., Exp. Br. Res. 54: 390, 1984. Franceschini, N. Neuroscience. Res. Suppl. 2: 15, 1985). The functional diagram of this microsystem has highligted a fine-grained network of *facilitatory controls* that operate in both directions -- preferred and non-preferred (Franceschini N. et al. in "Facets of Vision" ibid., 1989). We have now combined various sequences of stimuli such as pulses and/or steps (of light and/or darkness) on the two photoreceptor cells and determined the dynamic properties of the various filters that make up the various branches of a single E.M.D. Of particular interest is the preprocessing of signals which makes use of a nonlinear high-pass filter having a rectifying stage. Moreover this kind of filter is present on two E.M.D.'s that act in parallel to detect the motion of the leading edge and trailing edge of an object, respectively.

511.7

TRANSMITTER GATING, OPPONENCY, AND FACILITATION IN ELEMENTARY VISUAL MOTION DETECTION.
H. Öğmen* [1], S. Gagné* [2] (SPON: C. Harnois). [1]Dept. of Elect. Eng., Univ. of Houston, Houston, TX 77204; [2] Dept. of Elect. Eng., Université Laval, Ste-Foy, Québec, Canada, G1K 7P4.

Visually guided behavior of the fly involves detection of motion over short distances through directionally selective elementary motion detectors and integration of the outputs of these detectors over larger fields. Neurophysiological data support that large field integration and initiation of motor commands take place in the third optic ganglion (lobula complex), and the elementary directional motion detection in the second optic ganglion (medulla). The elementary motion detectors receive their inputs from a class of cells located in the first optic ganglion (lamina). Grounded on electrophysiological and behavioral data our model attempts to identify neural correlates of motion detection in this visual system. It comprises a first stage of processing based on the gated dipole model. The gated dipole incorporates depletable chemical transmitters gating input signals and lateral opponency between immediate neighbors through fast competition. The gated dipole model is used to synthesize sustained units and on-off units of lamina. The following process is lateral and directionally selective facilitation between sustained and on-off units to give rise to directionally selective elementary motion detection (in medulla). Finally, the outputs of elementary motion detectors are combined through complex receptive field profiles to feed motion sensitive cells of the lobula. Analysis and simulations of the model show that it is in good agreement with experimental data.

511.8

IN FLIES, MOTION-SENSITIVE NEURONS ARE POSTSYNAPTIC TO MULTIPLE REPRESENTATIONS OF SINGLE VISUAL SAMPLING POINTS: ANATOMICAL EVIDENCE FOR ELEMENTAL MOTION DETECTORS
Jong-Kyoo Lee and Nicholas J. Strausfeld. ARL Div. Neurobiology, Univ. of Arizona, Tucson, AZ 85721.

Intracellular recordings from *Calliphora* suggest that lobula plate wide-field motion-sensitive neurons (VS, HS) receive information about motion and direction from color-insensitive elemental motion detectors (EMDs) that, theoretically, should connect adjacent receptor channels [Egelhaaf M, Hausen K, Reichardt W, Wehrhahn C (1988) Trends Neurosci 11:351-358]. Our comparative Golgi analysis of Diptera, Lepidoptera, Coleoptera, and Hymenoptera, have identified analogous small-field retinotopic neurons (termed bushy T-cells) arranged as quartets in each retinotopic column projecting to wide-field neurons in the lobula plate, or an equivalent region in bees. This organization, which appears to have been conserved evolutionarily amongst different holometabolous orders, suggests that bushy T-cells may play a central role in ubiquitous optomotor reactions if they provide appropriate connections between adjacent retinotopic channels [Torre V, Poggio T (1980) Proc Roy Soc Lond B 202:409-416]. We have examined synaptic organization in the dipteran *Sarcophaga bullata*, at the level of wide-field motion-sensitive elements in order to determine if bushy T-cells provide their exclusive presynaptic elements, thus being obligatory components of EMD's. Serial sections show that while T-cells contribute the most prominent inputs to wide-field motion-sensitive neurons in the lobula plate, the latter also receive other types of neurons indicating that the inputs onto VS and HS neurons involve more types of retinotopic neurons than hitherto envisaged. Supported by NSF grant BNS-8719315.

511.9

DESCENDING NEURONS ASSOCIATED WITH WIDE-FIELD MOTION-SENSITIVE OPTIC LOBE AFFERENTS SEGREGATE TO NECK AND FLIGHT MOTOR NEUROPIL AND RESPOND TO PANORAMIC CUES.
Wulfila Gronenberg and Nicholas J. Strausfeld. ARL Div. Neurobiology, Univ. of Arizona, Tucson, AZ 85721.

Neuroanatomical studies of Diptera suggest that wide- and small-field neurons from the optic lobes segregate out to two distinct populations of descending neurons (DNs) connecting the brain with motor neuropils in thoracic ganglia. Golgi, cobalt, and Lucifer yellow staining in *Calliphora* demonstrate that a dorsal cluster of DNs receive terminals from large horizontal and vertical motion-sensitive neurons originating in the lobula plate. The terminals of these DNs visit dorsal neuropil of the pro-, meso-, and metathoracic ganglia which contain the dendritic trees of neck motor neurons involved in head yaw and roll (prothorax), and the dendrites of direct and indirect flight muscle motor neurons involved in flight stabilization (meso-, metathorax). We have recorded intracellularly from a variety of dorsal DNs. Recorded and dye-filled DNs characteristically respond selectively to the direction and orientation of panoramic motion and are color insensitive. The responses and morphology of these DNs contrast markedly with DNs in ventral neuropils that are associated with small-field visual interneurons originating in the lobula. As shown previously [Strausfeld NJ, Milde JJ (1988) Soc Neurosci Abstr 14:998], these ventral DNs are refractive to panoramic motion but are selective to other highly specific multimodal sensory contexts. The morphological and functional distinction of two parallel visual pathways from the compound eyes to motor centers suggests a segregation of form- and motion-sensitive pathways as far as the level of motor neurons. Supported by NIH grant EYO 7151-01

511.10

VISUAL OPTICS PREDICT NEURAL CONNECTIONS IN A PRIMITIVE DIPTERAN EYE.
S. R. Shaw, Life Sciences Centre, Dalhousie University, Halifax, Nova Scotia, Canada, B3H 4J1.

In pursuit of the nature of evolutionary change in the dipteran nervous system, little attention has been paid to changes in the optical configuration and associated neural wiring of the compound eye. All of the advanced brachyceran subgroup possess asymmetric arrays of photosensitive rhabdomeres R1-R8 (review: Shaw, S.R., in Facets of Vision, eds. D.G. Stavenga and R.C. Hardie, Springer, 1989); all but R3 align optically along the three natural, major (MA) axes of the facet array. In the primitive family closest to the brachyceran common ancestor, Anisopodidae, the rhabdomere pattern is different and more symmetrical. Quantitative analysis in vivo of several optical parameters shows that pairs of rhabdomeres R1-R6 align accurately at 30° to MA, along the minor axes (MI) of the facet array (mean misalignment 0.3° ± 2.6 sd, 9 eye maps). The measured interrhabdomere/interommatidial angle ratio (1.64 ± 0.22) is not significantly different from that required theoretically (1.73) for proper superposition of images along MI. Optimal functioning is anticipated when the axons of one ommatidium diverge to next-but-one cartridges along MI in the lamina, in contrast to the situation found even in the most primitive Brachycera. Electron microscopy supports extension of homologies to R1-R8 of anisopodids, and is being used to trace the axonal projection. Supported by grant A9593, NSERC.

511.11

PRESYNAPTIC SIGNAL PROCESSING AND FIELD POTENTIALS SHAPE THE RESPONSES OF FLY MONOPOLAR NEURONS.
M. Weckström * and S.B. Laughlin*. (SPON: K.Djupsund) Dept. of Zoology, Univ. of Cambridge, Downing St. Cambridge CB2 3EJ, UK.

Large monopolar cells (LMCs) are major second order neurons in insect eyes. Their transient response to retinal illumination codes intensity changes and rejects background signals. This behaviour is produced by the transient activation of photoreceptor-LMC synapses (Laughlin,S.B. and Osorio,D., J.exp.Biol.,in press; Weckström.M. et al., J.exp.Biol., in press). We investigated the mechanisms producing transient synaptic responses by applying single electrode voltage and current clamp techniques to photoreceptor axon terminals and LMCs. Extracellular potentials in the vicinity of the photoreceptor - LMC synapse were also studied by permeabilizing impaled LMCs with an intracellular injection of the detergent DMSO. The light responses of photoreceptor axon terminals show an initial fast spike-like depolarization, which is absent from somata, and which can be related to the response wave-form of LMCs. The lamina extracellular space has a standing voltage of ca. -30 mV. During illumination it depolarizes and reduces the pre-synaptic membrane potential driving transmitter release. Both these mechanisms shape the responses of LMCs.

511.12

EVOLUTIONARY PLASTICITY OF SYNAPTIC AFFILIATIONS IN THE DIPTERAN VISUAL SYSTEM.
D. Moore and S.R. Shaw. Dept. Bio. Sci., East Tenn. State Univ., Johnson City, TN 37614 and Life Sciences Ctr., Dalhousie Univ., Halifax, NS, Canada.

Golgi/EM comparisons between two families separated by >200 Myr of evolutionary divergence, the Stratiomyidae and Muscidae, have revealed that all of the neurons in the optic cartridges of the lamina neuropil are homologous and maintain relatively fixed positions with respect to one another (Shaw, Soc. Neurosci. Abstr. 14: 178, 1988). Thus development of the basic retinotopic unit, the lamina cartridge, appears to be invariant through evolutionary time. In contrast, EM investigations of the lamina cartridge neurons in Hermetia illucens, a representative of the archaic Stratiomyidae, demonstrate a variety of synaptic connectivities that are not found in the more-recently evolved Diptera (e.g. Musca). In Hermetia, L3 is missing inputs from R1-6 but receives inputs from R8 and L4. R8 is presynaptic to R7 and postsynaptic to R1-6; thus these three photoreceptor (R) subsystems are not independent, as they are in Musca. R1-6 provides inputs to L4 and L5 in Hermetia but not in Musca. C2, exclusively an efferent neuron in recently-evolved flies, receives afferent inputs from R1-6 in Hermetia. Less than 50% of the synaptic connections observed are found in common between Hermetia and Musca. Evolutionary progression in the nervous system has occurred via a vast reorganization of synaptic affiliations within a highly conserved neuronal set.

511.13

THE PUTATIVE ROLE OF ACTIN IN THE INNER SPACE OF THE OPEN-TYPE OMMATIDIA IN THE COMPOUND EYE. M.Järvilehto, P.Torkkeli*, R.Harjula*.Dept. of Zoology, Univ. of Oulu, 90570 Oulu, Finland.

The photoreceptor cell microvilli, the functionally significant parts of cells, are separated from each other by an extracellular, innerommatidial space (IOS), which composition and significance for the visual function were studied.

The prefixed (2% PA+0.2% GA/0.1-n phosph.buffer), frozen sections of the compound eye of the blowfly (Calliphora erythrocephala) were prepared by immuno-ultracryo-techniques, incubating by antibodies of α-tubulin, actin, filamin, spectrin and α-actinin for both EM- and immuno-fluorescence studies.

The immunocytological analysis shows that actin and filamin (not tubulin) are components of the IOS and not present in microvillar structures. The α-actinin is located just outside of the microvilli, whereas spectrin is present along the photoreceptor membrane inside IOS. We propose an actin based filament network to regulate optical adjustment of the ommatidia. This hypothesis is supported by the observation of deep pseudopupil deformation after treatment of ommatidia with 0.1 M dibutyryl-cAMP solution known to lead to a rearrangement of cytoskeletal actin.

511.14

CORRELATION BETWEEN INCREASE IN VISUAL SENSITIVITY AND BIOLUMINESCENT FLASHING ACTIVITY IN NIGHT-ACTIVE FIREFLIES. Abner B. Lall. Department of Zoology, Howard University. Washington D.C. 20059

Threshold sensitivity of the compound eyes was monitored over 18-20 hrs by recording ERGs among different species of fireflies. In dark-active fireflies of genus Photuris. visual sensitivity increased dramatically (1000-10,000 X) between 1830-2100 hrs, just prior to their initiation of flashing activity at night. This increase in sensitivity occurred only when the insects were maintained under strict natural ambient illumination conditions. When fireflies were kept in total darkness during morning daylight hours, the increase in sensitivity was not synchronized with evening hours. A change in threshold of visual sensitivity was not found in selected species which restrict their flashing activity to a short period at twilight or in non-bioluminescent day-active lampyrids.

NEUROETHOLOGY IV

512.1

TRADEOFF BETWEEN ECHO CROSSCORRELATION-PEAK DELAY AND TIME DELAY IN TARGET RANGING BY BIG BROWN BATS. W. M. Masters, Dept. of Zoology, Ohio State Univ., Columbus, OH 43210.

If asked to choose the nearer of two electronically simulated "phantom" targets in a two-alternative forced-choice (2AFC) task, big brown bats (*Eptesicus fuscus*) perform well if the simulated "echoes" are models of their own calls (range discrimination threshold about 1 cm at a target distance of 80 cm), but do poorly if the same echoes, reversed in time, are used (threshold typically >20 times larger) (Masters and Jacobs, unpub.). Clearly, the time-frequency structure of the echo is relevant to the bat, indicating that *Eptesicus* uses some type of matched-filter processing to determine echo arrival time. This finding is consistent with the idea that FM bats use crosscorrelation (matched filter, pulse compression) reception of echoes. Thus, a bat's estimate of echo delay (and, hence, of target range) will be affected not only by the travel time of the echo (travel delay), but also by the time of occurrence of the crosscorrelation peak (peak delay) of the echo. In other words, two echoes with the same travel delay (i.e., same actual range) but different peak delays should seem to be at different distances. To test this idea, I modified the time-frequency structure of echoes to give different peak delays and then, in a 2AFC task, determined the tradeoff between travel delay and peak delay in the bat's estimate of target range. Preliminary results with two bats suggest that the peak delay determined by cross-correlation gives a good approximation of the apparent echo-structure induced range shift perceived by the bat, again consistent with the possibility that FM bats use something closely akin to crosscorrelation processing of echoes for target distance.

512.2

BIG BROWN BATS USE SPECTRAL ECHO CUES FOR RANGE RESOLUTION J. Mogdans* and H.-U. Schnitzler* (SPON: K. Rudolph). Dept. Animal Physiology, University of Tuebingen, Morgenstelle 28, D-7400 Tuebingen, FRG.

Range resolution in echolocating bats refers to their ability to decide, whether an echo contains only one wavefront reflected from a single surface or two or more wavefronts from two or more closely spaced surfaces. This experiment examined performance and echolocation behavior of bats in a range resolution task.

Big Brown Bats (Eptesicus fuscus) were trained in a two alternative forced choice procedure to discriminate between electronically delayed versions of their echolocation calls simulating a one-wavefront echo (S^-) or two-wavefront echoes (S^+).

The ability of Eptesicus fuscus to discriminate a one-wavefront echo from two-wavefront echoes was limited to distinct internal time delays between the wavefronts of the double-wavefront echoes. Analysis of the two-wavefront echoes revealed periodic minima in the spectrum. Position and separation of the minima depend on the time delay between the two wavefronts. The occurance of minima within the frequency range of the first harmonic in the echo of the bats' echolocation calls correlates with the bats' ability to discriminate a one-wavefront echo from two-wavefront echoes.

From this finding we conclude that for range resolution Eptesicus fuscus uses spectral information of their sonar echoes rather than information about the arrival time of different wavefronts.

Supported by the Deutsche Forschungsgemeinschaft (SFB 307).

512.3

FURTHER ADAPTATION OF FM-FM NEURONS OF BAT'S AUDITORY CORTEX FOR RANGING. N. Suga, M. Kawasaki, and R.F. Burkard. Dept. of Biology, Washington Univ., St. Louis, MO 63130.

The mustached bat Pteronotus parnellii emits biosonar pulses consisting of 8 components: CF_{1-4} and FM_{1-4}. FM-FM neurons are tuned to a particular delay of echo FM_n (n = 2,3, or 4) from pulse FM_1 for processing target-range information. The FM signal is suited not only for ranging, but also target localization. If FM-FM neurons are directionally sensitive, however, this would greatly interfere with the encoding of range information. Electrophysiological studies indicate that the receptive fields of FM-FM neurons are large, covering the entire contralateral auditory field and medial half of the ipsilateral auditory field. FM-FM neurons are not suited for sound localization, and their best delays (best ranges) change little with changes in the direction of the echo source. The best azimuths of FM-FM neurons for echo FM_2, FM_3 and FM_4 are similar to those of peripheral neurons. However, the best azimuth for pulse FM_1 (2° front) is quite different from that of peripheral neurons (25° lateral). Since FM-FM neurons are conditioned by self-vocalized pulse FM_1 to process target-range information, their directional sensitivity to pulse FM_1 is an adaptation of these neurons for ranging. (Supported by PHS research grant NS17333).

512.4

MUSCIMOL APPLICATION TO THE BAT'S AUDITORY CORTEX DISRUPTS FINE FREQUENCY DISCRIMINATION OF BIOSONAR SIGNALS. H. Riquimaroux, S. J. Gaioni* and N. Suga. Dept. of Biology, Washington Univ., St. Louis, MO 63130.

The auditory cortex (AC) of the mustached bat, *Pteronotus parnellii*, has a highly specialized region, the DSCF area, which overrepresents the frequencies of Doppler-shifted echoes (about 61 kHz), and hence may play a role in the fine frequency discrimination necessary for detailed velocity measurements (Suga, 1984). We behaviorally tested this function of the DSCF area using reversible ablation with muscimol, a potent GABA agonist (Hikosaka *et al.*, 1985). Bats were trained on a discriminated shock avoidance task requiring a leg flexion response. The stimuli were trains of artificial pulse-echo pairs (tone bursts). For S+ the pulse and echo were the same frequency (e.g., 61.0 kHz), while for S- they were different frequencies (e.g., 61.0 kHz and 61.1 kHz). Following baseline behavioral training, we applied 0.1-0.2μl of 1μg muscimol/μl saline bilaterally to the DSCF area. The bats failed on previously successful discriminations of small frequency differences (e.g., 50 Hz), but succeeded on discriminations of large frequency differences (e.g., 2 kHz). Discrimination performance returned to baseline levels within 24 hours. These data indicate that the DSCF area is necessary for fine frequency discriminations involving biosonar signals. (Supported by AFOSR grant #87-0250.)

512.5

DELAY LINES IN THE INFERIOR COLLICULUS OF THE MUSTACHED BAT. T.Hattori* and N.Suga (SPON: Y.Fukami). Dept. of Biology, Washington Univ., St. Louis, MO 63130

To process target range information, the auditory system of the mustached bat, *Pteronotus parnellii*, creates arrays of FM-FM neurons which act as delay-dependent multipliers. The delay lines utilized by these neurons are as long as 18 ms. Studies on the lateral lemniscus and the brachium of the inferior colliculus indicate that delay lines are created in the inferior colliculus to a great extent (Kuwabara and Suga, ARO 1988). The aim of this study is to explore how the delay lines are created in the inferior colliculus.

Responses to tone bursts were recorded with microelectrodes from 1,117 collicular neurons of unanesthetized bats. In each subdivision of the central nucleus of the inferior colliculus, response latencies vary systematically from short to long along a ventrolateral-dorsomedial axis, i.e., along the lateral lemniscal fibers terminating within the inferior colliculus. The latency axis from 4 to 12 ms (a delay line of 8 ms) occupies about 2 mm distance across the anterolateral division. Since the diameter of myelinated lemniscal fibers in the inferior colliculus is about 1 μm, the conduction velocity of action potentials is expected to be about 6 m/sec, and the conduction time over 2 mm is calculated to be 0.33 ms. Therefore delay lines are predominantly due to synaptic delays and/or phasic on-inhibition.

(Work supported by PHS research grant NS17333)

512.6

CONNECTIONS OF FUNCTIONAL SUBDIVISIONS OF THE MUSTACHED BAT AUDITORY CORTEX. D. C. Fitzpatrick and N. Suga. Dept. of Biology, Washington Univ., St. Louis, MO 63130.

The auditory cortex (AC) of the mustached bat, Pteronotus parnellii, consists of multiple functional areas that process specific types of biosonar information. Targets of several of these areas were identified by combining physiological mapping with anterograde tract-tracing. The FM-FM area (a target range processing area) had the most extensive projections; labeled areas included:

Anterior cortex	AC	Subcortical telencephalon	Thalamus	Midbrain
1. Frontal	1. DF	1. Claustrum	1. MG	1. DLMT
2. Ant. Cing.	2. VF	2. Striatum	2. Other nuclei	2. SC
	3. VA	3. Amygdala		3. PMN
				4. IC

where DF,VF,and VA are distinct functional areas in the AC, MG=medial geniculate, DLMT=dorsolateral midbrain tegmentum, SC= superior colliculus, PMN=pontine motor nuclei, and IC=inferior colliculus.

Projections of the primary AC, the CF/CF area (a velocity processing area), DF and VF areas (additional range processing areas) were also identified, and included most of the structures identified above. The auditory cortex therefore contributes to multiple pathways involved in auditory sensory processing, vocalization control, and orientation of the head, ears and body. (Supported by PHS grants NS17333 and NS07057)

512.7

BICUCULLINE MODIFIES THE DELAY-TUNING OF FM-FM NEURONS IN THE MUSTACHED BAT. J.A. Butman and N. Suga (SPON: P.S.G. Stein). Dept. of Biology, Washington Univ., St. Louis, MO 63130.

The FM-FM area of the mustached bat's auditory cortex consists of an array of neurons which respond to biosonar pulse-echo pairs with specific echo delays, forming a map of target range. Facilitation occurs when pulse and echo responses coincide, so that neural delays must be introduced into the pulse response to compensate for the acoustic delay of the echo. Integration of pulse and echo responses occurs in the MGB where delay tuning is generated. Long best delay neurons receive inhibition which may be responsible for creating the appropriate neural delay. In order to examine the role of GABAergic inhibition in determining the best delay as well as for sharpening delay tuning, GABA and bicuculline methiodide (BMI) were iontophoretically applied to FM-FM neurons in both cortex and thalamus while recording from single neurons with multibarreled carbon-fiber electrodes.

In over half of cortical FM-FM neurons, BMI increased the width of the delay tuning curve while GABA sharpened this curve. The increases ranged from 20% to 100%. This suggests that cortical inhibition modifies the delay tuning generated in the thalamus. In the MGB, BMI also increased the delay tuning width. One class of MGB neuron showed a dramatic shift in best delay from long best delays to short best delays. This indicates that inhibition vetoes short latency responses creating the neural delays required to generate long best delay neurons. (Supported by NRSA Medical Scientist GMO7200 and PHS grant NS17333)

512.8

REPRESENTATION OF TARGET RANGE IN THE DORSOLATERAL MIDBRAIN TEGMENTUM OF THE BIG BROWN BAT. S.P. Dear and N. Suga. Dept. of Biology, Washington Univ., St. Louis, MO 63130.

For echolocation, the big brown bat, *Eptesicus fucsus*, emits orientation sounds consisting of two or three FM harmonics. Delay-tuned neurons in the intercollicular nucleus of this bat show a facilitative response to a synthesized pulse-echo pair, each containing three harmonics (Feng et. al., Science, 202:645-648, 1978). Our focus is to identify the essential elements for facilitation using single FM harmonics.

In unanesthetized bats, responses to pairs of linear FM sounds or pure tones were recorded using carbon-fiber microelectrodes. Best delays of delay-tuned neurons ranged from 0.5 ms to 28 ms and were systematically distributed from rostral (short delay) to caudal (long delay). Four-fifths of these neurons responded selectively to single harmonic pairs (FM_3-FM_1, etc) and one-fifth to multiharmonic pairs ($FM_{1,2}$-FM_3, etc). There was a high correlation between facilitation latency and best delay, r=0.96, but a poor correlation between pulse or echo alone latency and best delay, r=0.06 and r=0.47, respectively, suggesting that delay lines may not be the mechanism creating delay-tuned neurons in this bat. In order to identify the region containing delay tuned neurons, HRP was injected in several animals. The injection site was always located dorsal and caudal to the medial geniculate body, and ventral and lateral to the superior colliculus. Hence, we use the descriptive term: dorsolateral midbrain tegmentum. This region is located lateral and cadual to the intercollicular nucleus (Robards et. al., J. Comp. Neur., 170:499-524, 1976) .

(Work supported by PHS grant NS17333 and PHS grant NS07057)

512.9

PHARMACOLOGICAL DIFFERENTIATION OF CONTACT CALLS IN ADULT MALE SQUIRREL MONKEYS. J.C. Harris and J.D. Newman. Lab. of Comparative Ethology, NICHD, NIH, Bethesda, MD, 20892.

The isolation peep and twitter are acoustically distinct vocalizations of the squirrel monkey (Saimiri). They are grouped into the same functional category, "contact calls," due to their association with the establishment or maintenance of contact between familiar conspecifics. Peripheral administration of appropriate doses of the opiate receptor antagonist naloxone and the alpha2-adrenoreceptor yohimbine both increase production of the isolation peep in adult males visually and acoustically isolated from conspecifics during 15-minute sessions (Harris and Newman, 1987; 1988a). The combined administration of these 2 drugs increases production of the twitter under the same testing conditions (Harris and Newman, 1988b). To further understand the mechanisms involved in the neurochemical regulation of these vocalizations, we performed additional analyses on the relationships between drug-related changes in production of these 2 call-types, changes in locomotor activity while vocalizing, and the time of peak vocalizing. Drugs and their doses were: naloxone HCl (0.4 mg/kg), yohimbine HCl (0.05, 0.2, 0.4 mg/kg) and the combination of naloxone (0.4 mg/kg) and yohimbine (0.2 mg/kg). Significant differences in the differential production of isolation peeps and twitters were found for 2 drug treatments: 0.05 mg/kg yohimbine resulted in increased isolation peep production but no change in twitters, while the naloxone/yohimbine combination produced the opposite effect. Significant relationships between locomotor activity, peak time of vocalizing, and production of the two vocalizations occurred over all treatments. With respect to the isolation peep, level of locomotor and vocal activity were positively correlated, and more calls were produced early in a 15-minute session. With respect to the twitter, level of locomotor and vocal activity were negatively correlated, and the calls occurred relatively more frequently late in the tests. No significant relationships existed between locomotor or peak calling activity and the single vocal category of "contact calls" consisting of both isolation peeps and twitters. These results indicate the value of utilizing bioacoustic information in pharmacological studies of vocal behavior, and suggest a differentiation of the neurochemical mechanisms regulating production of 2 contact call sub-types in squirrel monkeys.

512.10

TYPE A AND B MONOAMINE OXIDASE INHIBITORS HAVE DIFFERENTIAL EFFECTS ON THE VOCAL AND MOTOR BEHAVIOR OF SOCIALLY SEPARATED SQUIRREL MONKEYS. J.D. Newman, J.T. Winslow, and D.L. Murphy. Lab. of Clinical Science, NIMH, and Lab. of Comparative Ethology, NICHD, NIH, NIHAC, Poolesville, MD, 20837.

Squirrel monkeys (*Saimiri*) emit a stereotyped vocalization, the isolation peep, when separated from familiar conspecifics. Previous work has demonstrated that primate vocalization is a sensitive measure of drug effect, providing a potential tool for examining the brain mechanisms controlling emotional behavior. We studied the acute effects of monoamine oxidase inhibitors L-deprenyl (0.5-5.0 mg/kg), clorgyline (1.0-5.0 mg/kg), and milacemide (100-400 mg/kg) on the behavior of male squirrel monkeys during brief (15 min) social separations beginning 60 min after subcutaneous drug administration. Monkeys were tested once per week until a stable baseline frequency of isolation peep calling was established. Scheduling of subsequent administration of drugs depended on reestablishing baseline calling rates, typically a minimum interval of 14 days between drug tests. All tests were videotaped for subsequent sonographic analysis of vocalizations, and computer assisted scoring of the frequency and duration of an exhaustive catalogue of other behaviors. All three drugs selectively reduced the rate of calling during social separation at doses which did not affect time spent in locomotion, nor the frequency of vigilance-checking. Deprenyl and milacemide, but not clorgyline, produced concurrent decreases in locomotion at the higher doses tested. The data demonstrate the utility of the social separation paradigm for the evaluation of drug effects. The data also suggest that MAO-A inhibitors may selectively affect vocal behavior, while MAO-B inhibitors may be more generally involved in arousal. This is consistent with previous reports of noradrenergic and serotonergic mediation of separation distress in rodents and non-human primates.

512.11

Development of tactile righting reflexes in the marsupial Dasyurus hallucatus. S.M. Pellis, V.C. Pellis and J.E. Nelson*. Dept. Psychol., Univ. Florida, Gainesville, FL 32611 and Dept. Zool., Monash Univ., Melbourne, Australia 3168.

During fetal development in the cat, tactile righting reflexes precede vestibular righting relexes (Windle, W.F. and Fish, M.W., J. Comp. Neurol. 54: 85-96, 1932). However, the "tactile system" as it pertains to righting behavior, is not one, but three distinct systems. In this study, the order of appearance and maturation of these tactile righting systems are described and analyzed for the pouch young of a carnivorous marsupial. The first form to appear was trigeminal righting, in which tactile input on the face can trigger rotation to prone. Next to appear was asymmetrical body contact triggering righting of the hindquarters, and last to appear was asymmetrical body contact triggering righting of the forequarters. The first form of righting appears at about 40 days postnatally, with all three maturing to the adult form by about 80 days.

512.12

THE COMPARATIVE NEUROBIOLOGY OF AFFILIATIVE BEHAVIOR AND SOCIAL BONDING: PRAIRIE AND MONTANE VOLES AS A MODEL SYSTEM. L. E. SHAPIRO,* C. M. LEONARD, C. E. SESSIONS*, AND T. R. INSEL. (SPON: P.D. MacLean). Section on Comparative Studies of Brain and Behavior, Lab. of Clinical Science, NIMH, Poolesville, MD 20837 and Dept. of Neuroscience, University of Florida College of Medicine, Gainesville, FL 32611.

The comparative, neurobehavioral analysis of interspecies differences represents a useful approach for investigating fundamental brain/behavior relationships. Prairie and montane voles are ideal for comparative analyses. Although closely related phylogenetically and morphologically, these two species display marked differences in social organization in the wild. Field data indicate that prairie voles are monogamous and live in extended family groups, whereas montane voles are polygamous, solitary, and females abandon their young at about 2 weeks after birth. Laboratory data have also recorded species differences in such affiliative processes as contact-proneness, pair bonding, and parental behavior (e.g., Shapiro et al., in press). The aim of the present research is to delineate the neural mechanisms which underlie species differences in affiliative behavior.

To explore species differences in social bonding and attachment processes during development, pups 5-10 days of age were placed in a temperature controlled plexiglass chamber and ultrasonic isolation calls were recorded during 2 min of social separation. Prairie voles vocalized at a mean rate of 138.4 ± 15.0 calls/2 min (N=28); montane vole calling rate was 27.9 ± 6.3 calls/2 min (N=26). Consistent with these results, circulating levels of plasma corticosterone increased significantly over baseline in prairie vole pups following 30 min of separation; corticosterone levels in montane vole pups remained unchanged following separation.

We are currently analyzing cytoarchitectonic differences in the cingulate cortex and the medial preoptic area. In addition, using *in vitro* receptor autoradiography, we are describing the distribution of oxytocin, opiate, CRF, and benzodiazapine binding sites in the two species. These studies represent preliminary efforts to elucidate the neural mechanisms that have been fine-tuned by natural selection to mediate the contrasting forms of social bonding displayed by these species.

512.13

EIGHT ARM RADIAL MAZE PERFORMANCE AND SOME RELATED BEHAVIORAL CHARACTERISTICS OF THE MEADOW VOLE, *Microtus pennsylvanicus*. G.C. Teskey, K.-P. Ossenkopp, N.K. Innis* and F.H. Boon*. Dept. Psychology, Univ. Western Ontario, London, Ontario, Canada N6A 5C2.

Eight arm radial maze performance was assessed in common meadow voles, *Microtus pennsylvanicus*. Based on maze performance and behavior patterns, voles were grouped into 3 categories; non-runners, strict-algorithmic runners and non-algorithmic runners. Some additional behaviors examined were performance on an interruption maze task, on a passive avoidance task, and spontaneous locomotor behavior in an automated Digiscan apparatus.

Relative to the other 2 groups, the strict-algorithmic runners were more active on measures of horizontal and vertical activity, and average speed and number of movements. On the passive avoidance task the non-runner voles showed much slower extinction of the task than did the other two groups. These results indicate that differences in eight arm radial maze performance in voles are a function of certain characteristics of spontaneous locomotor activity and are also reflected in performance on other tasks. (Supported by NSERC).

512.14

FOOD CONTAINING 6-MBOA IS PREFERRED BY FEMALE MICE AND VOLES. Randy J. Nelson, Department of Psychology, The Johns Hopkins University, Baltimore, MD 21218.

The plant compound, 6-Methoxybenzoxazolinone (6-MBOA), induces mid-winter breeding in natural populations of microtine rodents. 6-MBOA directly stimulates ovarian and uterine growth in laboratory tests, but in contrast to field data, does not directly affect males. It is not apparent how the small changes in female reproductive organ mass are related to the induction of winter breeding in populations of rodents fed 6-MBOA. In addition to its direct effects, 6-MBOA might indirectly stimulate reproduction by increasing food intake and subsequent body mass. This hypothesis requires animals to detect and prefer food containing 6-MBOA. Female prairie voles (Microtus ochrogaster) and CF-1 mice (Mus musculus) were provided a choice between specially milled food to which 0 or 50 μg/g of 6-MBOA was added. Daily food intake was measured for 10 consecutive days. Both species consumed more food containing 6-MBOA than food devoid of 6-MBOA. These results indicate that rodents detect and prefer 6-MBOA in food.

512.15

EARLY LESIONS PRODUCE DISSOCIATIONS OF THE ANTI-PREDATOR AND ORIENTING FUNCTIONS OF SUPERIOR COLLICULUS S.L.Ayres* & G.E.Schneider, Dept. Brain & Cogn. Sci., Massachusetts Institute of Technology, Cambridge, MA 02139.

Lesions of the SC in adult Syrian hamsters can abolish both turning movements (orienting) and anti-predator responses (escape movements, freezing) to visual stimuli (Schneider '67,'69,'79; Merker '80). We have found that early brainstem lesions can produce three different types of dissociations between these two types of responses. 1) *Supernormal* escape responses, with anomalous lack of habituation, can result from early ipsilateral thalamic lesions (N=3) with a spared or regenerated optic tract; orienting to seeds in these animals is intact. Early knife cuts across the brachium of the SC (BSC) have also produced some enhancement of escape responses (N=9). 2) A *loss* of escape responses with some sparing of orienting occurs in hamsters with early bilateral ablations of the superficial layers of SC (N=9). In these cases, the retina projects to remaining intermediate gray layer tissue. 3) Spared escape responses as well as turning in the wrong direction are found to depend on a re-crossing tectal bundle of optic-tract axons, after early unilateral lesions of the SC (N=7).

We conclude that separate tectal cell groups must control the two types of response, and that anti-predator responses may be subject to modulation by neural pathways that can be enhanced by early diencephalic lesions. (Supported by NIH grants EY00126 and EY02621.)

512.16

BEHAVIORAL CHARACTERISTICS OF AGGRESSIVE-DOMINANT AND SUBORDINATE MALE MICE. L.A. Hilakivi, M.J. Durcan and R.G.Lister. Lab. Clin. Stud., DICBR, NIAAA, Bethesda, MD 20892.

The behavioral characteristics of aggressive-dominant and subordinate male NIH Swiss mice were investigated. The aggressive-dominant mice were either 1) those which inflicted obvious bite marks on other mice in their cage but were not affected themselves (called alpha, n=18), or 2) those who attacked an intruder but were not constantly biting their cage mates (called attacker, n=4). The other mice in the study were 3) those housed with an alpha mouse; these mice had bite marks on their backs and tails (called subordinate-1, n=41), 4) those housed with an attacker mouse; these mice had no bite marks (called subordinate-2, n=28), and 5) those housed in cages in which no alpha or attacker mice could be identified (called control, n=30 and n=33). The behavioral tests used were Porsolt's swim test, a putative model of depression, the plusmaze test of anxiety, and the holeboard test of exploration and locomotor activity. Both alpha ($p<.01$) and attacker mice ($p<.05$) spent less time immobile in the swim test than their respective control mice. Subordinate-1 ($p<.05$) and subordinate-2 ($p<.01$) showed lengthened immobility in the swim test as compared to their controls. Because alpha and attacker mice differed in a similar manner from subordinate and control mice, the chronic fighting within the cage may not be responsible for the behavioral differences in the swim test. The swim test has been suggested to reflect animal's response to stress. If so, our data imply that aggressive-dominant mice may be able to cope with stressful situations, whereas subordinate mice may be prone to give up.

512.17

NEW DEVELOPMENTS IN FOOD CARRYING AND HOARDING IN THE RAT: SENSORY-MOTOR EVENTS AND NEURAL CONTROL . I.Q. Whishaw, I.A Tomie* and S.D. Oddie*, R.K. McNamara* & T. L. Harris*. Dept. Psychology, Univ. Lethbridge, Lethbridge, Alberta, Canada, T1K 3M4.

Foraging rats display different motor behaviors depending upon the size of pellets of food that they encounter. Small food pellets (20-45 mg) are swallowed, medium food pellets (74-190 mg) are held in the forepaws to be eaten, and large food pellets (>300 mg) are carried (hoarded) to available enclosures. The motor patterns associated with foraging also vary: when approaching food the rats use a stalking walk or trot, interrupted with pauses, but when carrying food they gallop. If hoarding is frustrated by blocking the home cage entrance, motor behavior is still still influenced by food pellet size: the rats dodge the food source with the pellet before eating it and dodge distance increases with food pellet size. The various motor behaviors associated with foraging can be differentially modified by environmental change as well as by central nervous system manipulations. For example, swallowing and eating are influenced by the size context in which a food pellet is given but hoarding is not. Frontal and amygdala damage does not affect swallowing and eating but after the former damage food is treated as being smaller than it is while after the latter damage food is treated as being larger than it is. Hippocampal damage abolishes hoarding. The motor patters of hoarding can also be differentially influenced by pharmacological manipulation, i.e., anticholinergics or minor tranquilizers. Thus, the motor patters of hoarding provide a rich selection of movements with which to analyze sensory influences and neural control of foraging.

512.18

EFFECTS OF INTRATYMPANIC INJECTIONS OF SODIUM ARSANILATE ON SPONTANEOUS LOCOMOTOR BEHAVIOR IN RATS: EXAMINATION OF BEHAVIOR IN AN OPEN-FIELD AND THE AUTOMATED DIGISCAN SYSTEM. K.-P. Ossenkopp, A. Prkacin* and E.L. Hargreaves. Dept. Psychology, Univ. Western Ontario, London, Ontario, CANADA N6A 5C2.

Vestibular dysfunction was induced in Long-Evans rats by intratympanic injections of sodium arsanilate (atoxyl). Following a 1 week recovery period the rats were tested for labyrinthine integrity by examining the loss of the air-righting reflex and the righting reflex (by lightly holding a sheet of Plexiglas against the soles of the rat's feet). All animals were then given two 10 min open-field tests during which ambulation, rearing, grooming and defecation responses were recorded. A week later all rats were tested twice in the automated Digiscan apparatus for 30 minutes.

Rats with vestibular dysfunction exhibited significantly more open-field ambulation but fewer rearing responses (ps<.01). In the Digiscan apparatus the atoxyl injected rats displayed significantly less time rearing (p=.01), although number of vertical movements did not differ significantly from the control rats. Time per vertical movement was greatly reduced in the experimental rats (p<.001). (Supported by NSERC).

512.19

THE HYPOTHALAMUS OF THE RAT: A NEURAL BASIS OF BEHAVIOURAL DIFFERENTIATION. J.G. Veening, T.A.P. Roeling, L.M.G. Geeraedts, J.H. Lammers*, M.R. Kruk*, R. Nieuwenhuys. (SPON: European Neuroscience Association) Dept. of Anatomy & Embryology, University of Nijmegen, * Dept. of Farmacology, University of Leiden, The Netherlands.

Anatomy: In a series of careful mapping studies we have observed that every part of the hypothalamus may be unique in its combination of (1) cytoarchitecture, (2) myeloarchitecture, (3) localization of various (neuromediator-specified) components of the Medial Forebrain Bundle, and (4) the destination(s) of its efferent fibers. Many of these fibers descend into the brainstem "paracore" (Nieuwenhuys (et al.),'85,'89), and may play a prominent role in the natural or artificial elicitation of behaviour.

Behaviour: Specific behavioural responses can be elicited by electrical stimulation of different but partially overlapping parts of the hypothalamus in the rat (Lammers et al., '87,'88a,b). Self-grooming can also be induced by microinjection of kainic acid near the paraventricular hypothalamic nucleus (Roeling et al., in prep.). A detailed study of this and other "behaviourally defined" parts of the hypothalamus is now performed in order to specify the differences in the afferent and efferent "wiring" of the neural substrate for grooming, aggression and possibly other kinds of behaviour.

512.20

EFFECT OF STIMULUS INTENSITY ON DISCRIMINATION OF ODORANT MIXTURES BY SPINY LOBSTERS IN AN ASSOCIATIVE LEARNING PARADIGM. J.B. Fine-Levy and C.D. Derby. Dept. of Biology, Georgia State University, Atlanta, GA 30303.

Previously, we have shown that the Florida spiny lobster, Panulirus argus, can behaviorally discriminate between members of a set of four artificial odorant mixtures: crab, oyster, mullet, and shrimp. In our previous paradigm, each group of lobsters tested was conditioned to two concentrations (0.05 and 0.5 mM) of the conditioned stimulus in order that they attend to stimulus type rather than concentration. They were then tested with the same two concentrations of each of the nonconditioned stimuli. In contrast, the present experiments were designed to examine the effects of mixture intensity on inter- as well as intra-type mixture discrimination. This was accomplished by conditioning lobsters to only one concentration (0.5 mM) of shrimp mixture and testing them with three concentrations (0.005, 0.05, and 5.0 mM) of shrimp mixture and two concentrations (0.05 and 0.5 mM) of oyster mixture. Preliminary data suggest that lobsters conditioned in the present paradigm continue to show a robust discrimination of oyster mixture from the 0.5 mM shrimp mixture, as well as an impressive recognition of oyster mixture versus the nonconditioned shrimp mixture concentrations. These inter-type differences are significantly larger than the observed intra-type differences. (Supported by NINCDS Grant No. NS22225 and a Whitehall Foundation Grant.)

512.21

BEHAVIORAL IMPORTANCE OF THREE "L"-SHAPED AUDITORY INTERNEURONS WHICH CO-EXIST IN THE PROTHORACIC GANGLION OF THE CRICKET *ACHETA DOMESTICA*. G. Atkins, S. Atkins and J. Stout. Dept. of Biol., Andrews Univ., Berrien Springs, MI 49104.

The morphology of three auditory interneurons is described for *Acheta domestica*. Although L1, L2 and L3 share a similar gross structure there are differences which consistently identify each of the neurons and which are always correlated with certain response properties. The coexistence of L1, L2 and L3 is confirmed by staining all three in a single hemiganglion.

In previous work we reported a technique for evaluating the behavioral role of identified auditory interneurons in the cricket. Individual neurons were photoinactivated with blue light after being filled with Lucifer Yellow. Orientation after photoinactivation was compared to pretests and differences in the behavior were attributed to elimination of the killed neuron. We have repeated these unilateral experiments using a non-compensating treadmill for the behavioral testing which allows for continuous tracking by the cricket for longer periods of time and have duplicated and extended our earlier results. Of particular interest, killing an ON1 results in angular deviations which are intensity and syllable period specific (threshold for deviation is the same as the spiking threshold of L3 but well above the threshold of ON1).

To test the necessity of a mirror-image pair of interneurons one would have to photoinactivate both members in the same animal. Since there are three pairs of "L"-shaped interneurons which are closely associated with each other in the anterior Ring Tract, the chances of a bilateral kill of the left and right member of one type is small. To avoid this problem we have begun to examine phonotaxis in "one-eared" (one tympanum waxed) crickets. Unilateral killing of a neuron on the intact side of a "one-eared" cricket allows us to evaluate phonotaxis with both members of a given pair inactive.

512.22

LOCUSTS COMPUTE DISTANCE BY MOTION PARALLAX. E. C. Sobel, Inst. of Neurological Sciences, Univ. Pennsylvania, Philadelphia, PA 19104.

Before jumping to a target locusts induce motion parallax by side to side "peering" movements (Wallace, G. K., J. Exp. Biol. 36:512-525, 1958; Collett, T. S, J. Exp. Biol. 76:237-241, 1978). We presented 4th and 5th instar locusts (*Schistocerca Americana*) with vertical black rectangular targets at varying distances (subtending constant visual angle) which they jumped to when radiant heat on their perch increased slowly. Jump velocity increased as target distance increased suggesting that locusts have distance perception.

By electronically tracking the head position of the animal while it peered at a target, the target could be moved laterally during peers to create the proximal stimulus of a target closer or farther from the locust. Locusts were fooled by the artificial parallax and jumped with the same velocity toward targets at an illusory distance as they did to targets actually at that distance, demonstrating distance perception by motion parallax.

Locusts are sensitive to the absolute value but not the sign (direction) of motion parallax. When presented with paradoxical parallax (a target whose motion parallax describes a negative distance from the animal ie. behind the animal) locusts jump forward with the same velocity as if the target were located at the absolute value of the computed distance. Supported by MH17168

512.23

THE GROSS ANATOMY OF THE CENTRAL NERVOUS SYSTEM OF NAVANAX INERMIS (GASTROPODA; OPISTHOBRANCHIA). J.L. Leonard, Dept. of Zoology, University of Oklahoma, Norman, OK 73019.

In the course of recording from whole-nerves in freely-moving Navanax using cuff electrodes, I discovered that the available information on the anatomy of the central nervous system was not adequate to describe the recording site unambiguously. Although Murray (1971) described not only the roots but also the general target areas and functions of the major pedal nerves, there are branches of these nerves large enough to carry an electrode that have not been described. Also, except for that study, the information available is largely confined to discussions in the taxonomic literature of the nerve roots. Therefore, simple methylene blue staining, dissection, and photomicroscopy have been used to produce a more adequate description of the gross anatomy of Navanax. The major findings of the study concern a) finer branches of the pedal nerves, particularly P3c, P4 and P5; b) the distribution of nerves from the tail (abdominal and subintestinal) ganglia, including the abdominal and pallial nerves; and c) the presence of a variable number of large (>100um) white somata in the supraintestinal ganglion, along with a bundle of large white fibers running down the commissure to the abdominal ganglion. The color and location of these cells suggests a neuroendocrine function.

Supported by NIMH FIRST grant #BPN-2 #R29 MH43302-01 and OHRP #HRC-RRP-D-024.

INVERTEBRATE MOTOR FUNCTION

513.1

THORACIC INTERNEURONS OF THE VENTRAL GIANT SYSTEM EXCITE LEG MOTOR NEURONS OF MULTIPLE GANGLIA DIRECTLY AND VIA LOCAL INTERNEURONS. A.J Pollack* and R.E. Ritzmann. Dept. of Biology, Case Western Reserve University, Cleveland, OH 44106.

Our laboratory has been studying the wind mediated escape system of the cockroach Periplaneta americana as a model which will allow us to determine how populations of interneurons interact to control orientation behaviors. As part of this long term investigation, we have identified a large population of thoracic interneurons (type A TIs) which are excited by ventral giant interneurons (vGIs) over constant short latency connections.

Since this population of TIs is the point where sensory information from vGIs is integrated and presumably utilized to control motor activity, we wanted to examine the pathway from type A TIs to motor neurons both in the ganglion in which the TI originates and in distant ganglia. We used paired intracellular recording and dye injection techniques to map the motor pathway. Our data demonstrate that both leg motor neurons and local interneurons are excited by type A TIs over constant short latency connections. Thus, the motor neurons are activated by these TIs both directly and by an indirect pathway that may serve to better coordinate multiple motor activities. Connections were demonstrated both in the ganglion of origin for the TIs and in distant ganglia. To date all connections between TIs and motor neurons or local interneurons in more anterior ganglia have been excitatory, whereas all connections in more posterior ganglia have been inhibitory.

This work was supported by NIH grant NS 17411 to R.E.R.

513.2

EFFECTIVENESS OF AFFERENTS IN THE RECEPTIVE FIELDS OF SPIKING LOCAL INTERNEURONES OF THE LOCUST M.Burrows Department of Zoology, University of Cambridge, CB2 3EJ, England.

Local interneurones process mechanosensory signals from the legs of a locust and convert it into appropriate reflex movements that adjust posture and locomotion. The first stage in this processing is performed by spiking local interneurones which receive direct excitatory inputs from exteroceptive afferents and make inhibitory synapses with other spiking and nonspiking local interneurones, with intersegmental interneurones and with some motor neurones. Each interneurone is excited by a particular array of receptors that form part of its excitatory receptive field. Two questions about the organisation of these fields are posed. First, how effective is a sensory spike in evoking a spike in an interneurone? Second, are all receptors within a field equally effective? These questions have been answered by recording intracellulary from an interneurone and monitoring the spikes of many afferents in its receptive field. There is a gradient of effectiveness within a field. An afferent from a hair toward the edge produces small EPSPs that are unable to make the interneurone spike. Hairs in the centre of a field may, by contrast, evoke large EPSPs so that each afferent spike reliably evokes a spike in the interneurone. Supported by NIH grant NS16058 and by SERC (UK).

513.3

MOTOR NEURONS ARE ORGANIZED INTO GROUPS IN THE METATHORACIC GANGLION OF THE GRASSHOPPER. M.V.S. Siegler* and C.A. Pousman* (SPON: D.B. Morton). Dept of Biology, Emory Univ., Atlanta, GA 30322.

We have examined the morphology of CoS silver-intensified leg and wing motor neurons in the metathoracic ganglion of the grasshopper Schistocerca americana, in whole mounts and reconstructed serial sections. The motor neurons fall into stereotyped and invariant anatomical groups in the cortex of the ganglion. Within a group the cell bodies are clustered and the primary neurites enter the neuropil at the same point, forming a discrete bundle. The bundles are distinctive in shape and position, and can be reidentified in different individuals. Primary neurite bundles are separate from bundles of motor axons exiting or sensory axons entering the neuropil. The groups of neuronal cell bodies appear to be separated from each other by investitures of glial cytoplasm.

The motor neurons within an individual group contrubute axons to 1-3 peripheral nerves, and supply from 3-7 muscles. The motor neurons of a single muscle may fall into one group or separate groups. They do not appear to be mapped topographically by peripheral nerve or muscle innervated, or functionally by their involvement in a particular behavior. Instead, we suggest that the cortical organization of motor neurons reflects events early in the development of the nervous system.

513.4

PROPERTIES OF THE SMALL DORSAL UNPAIRED MEDIAN (DUM) NEURONS OF THE GRASSHOPPER. K.J. Thompson and M.V.S. Siegler*. Dept of Biology, Emory Univ., Atlanta, GA 30322.

We are interested in discovering the constraints on diversity of functional and morphological properties in the progeny of single neuroblasts. The DUM neurons in the grasshopper are the progeny of the single unpaired median neuroblast (Goodman & Spitzer. Nature 280:208, 1979). In the adult the DUM neuron group contains some 20 larger cell bodies (diameter 50-80um) roughly anterior to some 60-70 smaller cell bodies (15-25um). The large DUM neurons are octopaminergic efferent modulatory neurons (Evans & O'Shea. JEB 73:235, 1978). Only these larger neurons stain with Ag-S, consistent with the involvement of copper in octopamine biosynthesis (Wallace. J. Neurochem 26:761, 1976). In contrast, the small DUM neurons, but not the large ones, express GABA-like immunoreactivity suggesting that they may function as inhibitory neurons.

Intracellular electrophysiological and morphological techniques were used to investigate the members of the small DUM neuron group. We have found them to be broadly divided into two classes 1) local auditory interneurons, 2) intersegmental interneurons with diffuse branching patterns, that fire in association with voluntary movements of the animal. Both types have passively-conducted soma spikes (5-15mV) and display a variety of shapes, but are bilaterally symmetrical. Most project to the mesothoracic ganglion, or branch locally in the auditory neuropil.

513.5

TEMPERATURE EFFECTS ON LOCUST FLIGHT. J.A. Foster* and R.M. Robertson (SPON: L.Z. Wise). Department of Biology, Queen's University, Kingston, Ontario, K7L 3N6, Canada.

Thoracic temperature in flying locusts can be greater than 35 C but the effect of such temperatures on the properties and interactions of flight interneurons is unknown. This initial study characterized the relationship between thoracic temperature and wingbeat frequency in intact, tethered Locusta migratoria.

Locusts were flown in a wind tunnel for 1-3 hours while monitoring thoracic temperature and wing-beat frequency. At an ambient temperature of 24 C, the start-up stage of flight was characterized by an increase in both thoracic temperature and wing-beat frequency. At the transition from start-up to steady state flight there was a gradual decrease in thoracic temperature and wing-beat frequency to a level which was maintained for up to two hours. Artificial elevation of ambient temperature led to an increase in thoracic temperature and a corresponding increase in wing-beat frequency.

Previously it has been reported in Schistocerca that wing-beat frequency is independent of temperature. Our results suggest that in Locusta, wing-beat frequency is not independent of temperature but rather, wing-beat frequency varies with thoracic temperature during start-up and during steady state flight.

513.6

A NEUROANATOMICAL STUDY OF INTERSEGMENTAL INTERNEURONS IN THE LARVAL AND ADULT STAGES OF THE INSECT, MANDUCA SEXTA. T. M. Amos * and K. A. Mesce. Graduate Program in Neuroscience and Department of Entomology, Univ. of Minnesota, St. Paul, MN 55108.

The holometabolous insect, Manduca sexta, has been the focus of much research regarding the sensory and motor elements underlying various behaviors; much less is known about the identities and behavioral roles played by interneurons.

Many rhythmic motor patterns, including walking and ecdysis (the shedding of the old cuticle), are thought to involve intersegmental interneurons that assist in the coordination of neural networks residing in the segmental abdominal and thoracic ganglia. Intersegmental interneurons may also have a neuromodulatory function, for example, pterothoracic intersegmental interneurons determine whether the adult will display adult-specific or larval-like ecdysis behavior. Even the programmed death of particular abdominal motoneurons is influenced by putative intersegmental neurons descending from the pterothoracic ganglion.

To determine the numbers and locations of intersegmental interneurons present in the larva and adult, we constructed neuroanatomical maps of those neurons whose cell bodies originated in a given ganglion and whose processes (both ascending and descending) spanned multiple ganglia. Cobaltous-chloride (1-3%) or cobaltous-lysine (<100mM) was applied to the cut ends of ganglionic connectives at various anterior and posterior levels to stain the neurons. In the larva, sets of morphologically homologous neurons were found whose axons travelled through nine or more ganglia. In both the larva and adult, the numbers of intersegmental neurons per ganglion were found to be as few as one or two pair per ganglion; the cell bodies were often relatively large (40um) although quite small somata (<15um) were also observed. Maps of these neurons will be valuable in determining whether postembryonic neurogenesis plays any role in the production of the adult intersegmental interneurons.

513.7

MECHANISMS OF DEPOLARIZING SYNAPTIC INHIBITION AT THE GIANT MOTOR SYNAPSE OF CRAYFISH. D.H. Edwards, Biology Department , Georgia State University, Atlanta, GA 30303.

The crayfish giant motoneuron (MoG) is excited by giant interneurons through the electrically rectifying giant motor synapses and inhibited by depolarizing IPSPs from other interneurons. I have found three novel inhibitory mechanisms activated by postsynaptic depolarization that supplement the previously described GABA-mediated increase in membrane conductance: (i) depolarization reverse-biases the rectifying electrical synapse and so decreases excitatory synaptic current through it; (ii) it decreases the input resistance of MoG and so adds to the increase in postsynaptic membrane conductance, and (iii) it inactivates voltage-dependent sodium conductances and so raises the motoneuron firing theshold. All four mechanisms produce similar degrees of inhibition. Mechanism (i) is likely to operate at other rectifying electrical synapses in crayfish and elsewhere. Mechanisms (ii) and (iii) may operate at depolarizing inhibitory synapses in vertebrates and invertebrates, including those that produce primary afferent depolarization. Supported by NIH Research Grant NS26457.

513.8

PRE- AND POSTSYNAPTIC MECHANISMS OF GABAERGIC INHIBITION AT A CRAYFISH SENSORIMOTOR SYNAPSE. B.M.Rawat* and P.Skorupski* (SPON:B.Matthews). Dept. of Physiology, University of Bristol, Bristol, UK.

A non-spiking proprioceptive afferent (the T-fibre) from the base of a crayfish leg makes direct monosynaptic excitatory and disynaptic inhibitory connections with promotor motoneurones. The reflex expression of these alternative pathways is correlated with the phase of centrally generated motor activity and regulated by both pre- and postsynaptic factors. One factor may be GABAergic inhibition. GABA is an inhibitory neurotransmitter in the CNS of both vertebrates and invertebrates. Its actions are known to be mediated by two receptor subtypes, GABA-A and GABA-B. GABA-A receptors are coupled to a Cl^- channel and predominantly located postsynaptically. GABA-B receptors are not coupled to a Cl^- channel and are often found presynaptically (although both subtypes have been found pre- and postsynaptically).

Bath application of GABA to the isolated crayfish thoracic nervous system has both pre- and postsynaptic effects. GABA (at 10^{-4} M) depolarises the central terminals of the T-fibre with an increase in conductance. This effect can be reversed when the T-fibre is held depolarised at 10-15mV from rest. GABA (again at 10^{-4}M) hyperpolarises promotor MNs, with a similar increase in conductance. Both effects are reversible. Baclofen, a GABA-B agonist, (at 10^{-4} M) does not alter the membrane potential or input resistance of the T-fibre, but does hyperpolarise promotor MNs, though with a smaller rise in conductance. This effect is reversible and persists in the presence of the GABA-A antagonist picrotoxin (at 10^{-5} M).

These preliminary results suggest that in this crustacean system both classes of GABA receptor are present, though with a different pre- and postsynaptic distribution. This system, which has the advantage of being accessible, may be a useful model for the study of central GABAergic inhibition.

Supported by a SERC grant to B.M.H.Bush. B.M.Rawat is a SERC Scholar.

513.9

GABA MEDIATED INHIBITION OF FLEXION PRODUCING INTERNEURON AND FLEXOR MOTONEURON IN LOBSTER ABDOMEN. V. C. Kotak and C. H. Page, Nelson Biol. Labs, Rutgers Univ., Piscataway, N.J. 08855.

A population of flexion producing interneurons (f3FPIs) bilaterally excite flexor motoneuron f3. Since mechanical stimulation of the swimmeret inhibits the f3FPIs and f3, we tested the possible GABAergic nature of this inhibition. Bath application of 5μM picrotoxin produced a mixed response to swimmeret stimulation of IPSPs intermingled with a few spikes in both neurons. Amplitudes of their sensory evoked and spontaneous IPSPs also decreased. Higher picrotoxin levels (20μM) further reduced sensory evoked inhibition while increasing the spiking response. At higher concentrations (100μM) swimmeret evoked and spontaneous IPSPs were completely blocked, with f3FPI and f3 responding to swimmeret stimulation with a burst of spikes. The effects of picrotoxin were not reversible. Bath application of GABA (100μM-500μM) decreased amplitudes of swimmeret evoked IPSPs, and spontaneous spikes and IPSPs, by as much as 50% in f3FPI and f3. Nipecotic acid (500μM) enhanced the frequency of IPSPs in both cells. These results indicate that swimmeret evoked inhibition of f3FPI and f3 is produced by GABAergic interneurons.

513.10

WHITE NOISE ANALYSIS OF REFLEX STIFFNESS IN HERMIT CRAB ABDOMINAL MUSCLE. William. D. Chapple. Dept. of Physiology and Neurobiology, University of Connecticut, Storrs, CT 06269.

Previous measurements (Chapple 1985) of the increase in muscle stiffness produced by a stretch reflex in the abdominal ventral superficial muscle of the hermit crab, Pagurus pollicarus, using ramp stretches of the muscle indicated that the reflex produced only a modest increase in muscle stiffness. The present experiments were designed to confirm these results and to characterize the action of the stretch reflex in the frequency domain. Bandpass Gaussian noise with a maximum amplitude of 6% of muscle optimum length was used to perturb the muscle while force and medial motoneuron frequency were recorded. Fast Fourier transforms of length (input) and force (output) were used to calculate the frequency response spectral density function during a ten second noise sequence under three conditions: 1) entire abdominal nerve cord, 2) 4th ganglion alone, and 3) passive muscle. Both 1 and 2 had stiffness magnitudes twice that of the control (3), but did not modify the dynamics of the muscle.

513.11

CHARACTERIZATION OF THE ANTENNAL COMMON INHIBITORY MOTONEURON IN CRICKETS. B. Brunninger*, C. Allgäuer* and H.-W. Honegger. Inst. Zool., Technische Universität München, Lichtenbergstr. 4, D-8046 Garching, W.-Germany

In crickets, movements of the antennae are controlled by 7 muscles. Cobalt backfills from these muscles revealed 18 motoneurons, of which one, with its cell body in the medio-ventral deutocerebrum, was filled from 6 of the 7 muscles. Thus, this motoneuron sends axon collaterals to several muscles, similar to common inhibitory (CI) neurons in the thoracic ganglia.

Two lines of evidence support the idea that this antennal motoneuron is a CI. Firstly, electrical stimulation of one of these muscles initiated inhibitory potentials (ijp's) in the fibers of each of the other muscles, indicating that the axon collaterals shown anatomically are inhibitory in nature. Secondly, since GABA is known to be the common transmitter at inhibitory peripheral synapses in invertebrates, we employed a combination of cobalt backfills and GABA immunoreactivity to double stain this neuron. Cobalt backfills were done from one antennal muscle. Brains were fixed and embedded in paraplast. Alternate 8 µm sections were either silver intensified or incubated with rabbit GABA antisera. Reactive sites were visualized by the PAP procedure.

The neuron with its distinct cell body location in the medio-ventral deutocerebrum is the only antennal motoneuron labelled with both cobalt and GABA-antibody. In addition, one axon branching into the antennal nerves which innervate the target muscles of the double stained neuron showed GABA immunoreactivity. The results thus demonstrate that the antennal motor system contains one CI neuron.

513.12

NETWORK CONTROL OF FLIGHT IN THE DRAGONFLY W. Faller* and M.W. Luttges. Aerospace Engineering Sciences, University of Colorado, Boulder, Colorado 80309.

In order to understand the neural control of unsteady aerodynamics utilized in sophisticated flight, dragonflies have been studied. The interactions between sensory inputs and network properties that control the various flight modes are not well understood. To study the neural control algorithms responsible for the control of flight, extracellular multiple-unit recordings were obtained from the mesothoracic ganglion during a time period ranging from pre-flight through post-flight episodes. Classical and new multiple-unit analysis techniques, such as gradient analysis and correlation matrices, were performed in order to characterize the sensory, local, and global network control of flight. Changes were observed in the spatio-temporal interactions between groups of cells. Results indicated that variations occurred in the neural activity patterns and interactions both at the local, and global level. These changes were responsible for the control of various phases in the pre-flight to post-flight regime. The significance of these global control algorithms as they relate to the network and sensory control of flight is discussed.

513.13

SOMA POSITIONS AND NERVE PROJECTIONS IN THE BUCCAL SYSTEM OF *APLYSIA CALIFORNICA*. M.L.Scott*, P.J. Church, and M.D. Kirk. Dept. of Biology, Boston University, Boston, MA 02215.

To obtain more detailed information on the overall organization of the buccal ganglia and innervation of the buccal musculature, we have systematically backfilled each of the buccal nerves using $CoCl_2$ and are mapping the motor projections of these nerves using extracellular and intracellular recordings. Backfills of all buccal nerves (N1-N6 and CBC) show both ipsilateral and contralateral cell bodies, although soma positions are unique for each nerve. The size, number and positions of cell bodies suggest sensory and/or motor functions for particular cells projecting in respective nerves. The innervation experiments revealed unique motor projections for nerves N1 and N4-6. One unique result was obtained from N3 (salivary nerve) backfills; most stained processes formed a single bundle which looped near the ganglion and exited via N2. A few (1-5) of these processes separated from the bundle, entered the ipsilateral ganglion, crossed the commissure, and exited via contralateral N2. This result, combined with the fact that very few somas stained from N3, suggests that the salivary gland may be under substantial direct peripheral control from the gut. With these studies we can further define functional groupings of neurons in the buccal ganglia. Supported by NIH grant NS24062.

513.14

AN IDENTIFIED POPULATION OF SEROTONERGIC NEURONS ENHANCES NEUROMUSCULAR TRANSMISSION IN THE PARAPODIA OF APLYSIA BRASILIANA. D.R. McPherson and J.E. Blankenship, U. of Texas Medical Branch, Galveston, Texas 77550

The marine mollusc Aplysia brasiliana swims by rhythmic flapping movements of its parapodia. Using a reduced preparation we have studied the function of two types of pedal ganglion neuron that innervate the parapodia. One type causes discrete movements. Simultaneous intracellular recordings from these cells and muscle fibers reveal one-for-one junction potentials (e.j.p.'s). These persist in artificial seawater containing 5 x normal [Ca^{+2}], suggesting a monosynaptic connection. Most motoneurons are phasically active during fictive swimming.

The second type of efferent neuron is also phasically active during fictive swimming but has no detectable direct motor effect. These cells are termed parapodial opener phase cells (POP cells) based on their activity pattern. The activity of POP cells greatly increases the tension and the rate of relaxation recorded from parapodial muscle in response to motoneuron firing. Intracellular recording from muscle fibers after POP cell firing reveals an increase in e.j.p. amplitude. POP cells contain serotonin-like immunoreactivity, and bath-applied serotonin (10 µM) mimicks the POP cell effects. Supported by NIH, NS 27314, NS 07185 and NSF BBS 8711368.

513.15

INNERVATION OF THE ANTERIOR ARTERIAL SYSTEM OF *APLYSIA CALIFORNICA*. M.E. Skelton*, L.B. Buck* and J. Koester. Center for Neurobiology and Behavior and Dept. of Psychiatry, Columbia University, 722 West 168 Street, NY, NY 10032.

In *Aplysia* swelling of the lips during food-induced arousal and phasic shunting of blood flow during feeding movements are probably mediated by neurons in the head ganglia that innervate the distal part of the anterior aorta and its branches. The innervation of the posterior cardiovascular system, including the proximal part of the anterior aorta, has been reported previously. This study aims to identify neurons in the head ganglia that innervate the anterior aorta and to detail their interaction with cells involved in the generation of feeding behaviors and the control of the posterior cardiovascular system.

Experiments to identify the possible transmitter/modulator substances present in the anterior vessels showed that branches of the anterior aorta supplying the lips were rich in axons exhibiting yellow and blue glyoxylic acid induced histofluorescence, suggesting serotonergic and dopaminergic innervation. These lip arteries also contained varicose fibers immunoreactive to small cardioactive peptide B (SCP_B), FMRFamide and serotonin antibodies but contained no buccalin, peptide histidine isoleucine (PHI) or L11 peptide-like immunoreactivity.

Recording perfusate pressure of isolated lip artery preparations while applying putative transmitters/modulators showed that acetylcholine, serotonin, dopamine, $R15\alpha_1$ and $R15\alpha_2$ peptides (derived from alternative splicing of RNA encoding an R15 polyprotein) caused constriction of the vessels, whereas SCP_B and FMRFamide caused dilation. The search for candidate motoneurons/modulators of the anterior vascular system is being concentrated on cells that contain these vasoactive substances.

513.16

CARDIAC INNERVATION IN THE NUDIBRANCH, ARCHIDORIS MONTEREYENSIS. B.L. Wiens* and P.H. Brownell (SPON: J. Presson) Dept. of Zoology, Oregon State Univ. Corvallis, Or. 97331

The heart of Archidoris montereyensis is regulated by a small number of powerful motor neurons. We have identified five cardiac motor neurons in the CNS. Two motor neurons in the pleural ganglion, an excitor and an inhibitor, have particularly potent actions on the heart. Low levels of spontaneous activity in either cell significantly alter the amplitude and rate of ventricular contractions. Three motor neurons in the visceral ganglion, one excitor and two inhibitors, are less effective in altering cardiac activity, although they exhibit actions similar to their counterparts in the pleural ganglion.

We are currently investigating the activity of molluscan cardioactive transmitters on an isolated preparation of the Archidoris heart. ACh decreases the amplitude, rate, and tonus of heart contractions in a dose-dependent manner with a threshold of 10^{-9}-10^{-8}M. 5-HT, with a similar threshold, excites the heart, predominantly increasing the amplitude of contraction. Two neuropeptides, small cardioactive peptide B and FMRFamide increase the amplitude and rate of contraction with thresholds of 10^{-8}-10^{-7} and $<10^{-9}$, respectively. Cardioactive fractions isolated from the nervous system of Archidoris contain both protease sensitive and insensitive components. Supported by a Sigma Xi Grant-in-Aid of Research.

513.17

A VIDEO-MICROSCOPIC STUDY OF THE LEECH FEEDING APPARATUS DURING INGESTION. C. M. Lent and D. Zundel*. Department of Biology, Utah State University, Logan UT 84322.

The stereotyped ingestive behavior of *Hirudo medicinalis* is characterized by pharyngeal peristalsis (Lent, C., *et al., J. Exp. Biol.* 137:513, 1988). Protracted biting by jaws during ingestion has been suspected, but the opacity of flesh and blood has prevented confirmation. Leeches are now known to ingest a transparent fluid (1mM Arg, 50mM NaCl, Elliot, E. *J. comp. Physiol.* **A** 159: 391, 1986) upon which *Hirudo* imbibed in describing the activity of their three jaws during ingestion. Fluid (~37°C) was poured into a cylinder, leeches bit on its Parafilm™ membrane, and exhibited movements and durations typical of blood meal ingestion. Rhythmic biting movements not only cut into the film, but persisted throughout the 30 minute ingestive period. Jaw movements are synchronized to the pharyngeal rhythm. Both rates begin at ~3 Hz and decay logarithmically until termination. The depth of jaw insertion increases during ingestion and saliva accumulates visibly on jaw cutting edges. The power stroke of the biting movements appears to be the adoral direction since its velocity exceeds the oral movement. Three to five seconds before the leech terminates ingestion, biting and pharyngeal movements cease completely. Supported by a grant (to C.M.L.) from the Willard L. Eccles Charitable Foundation.

513.18

IS THE EARTHWORM MEDIAL GIANT FIBER A PLATONIC NEURON? J.L. Johnson. Dept. of Physiology and Pharmacology, USD School of Medicine, Vermillion, SD 57069.

According to Llinas (Science, 242:1654, 1988) a platonic neuron serves as a simple threshold element and the output pattern primarily reflects the connectivity of synaptic inputs. The earthworm medial giant fiber (MGF) must generate a repetitive burst of action potentials in order to organize an effective head end escape response. Since the MGF is activated by sensory inputs, the question is, does the MGF contribute to the production of the physiologically effective discharge pattern? It is shown in this study that facilitation mechanisms operative within the MGF are capable of initiating a discharge rate which can lead to a maximally effective head end escape response. The facilitation effects exerted by the MGF which enhance its own responsiveness involve (1) the generation of local nodal action potentials, (2) self-stimulation, and (3) a substantial depolarizing afterpotential following a conducted spike. The ability to enhance the probability of occurrence of a physiologically effective discharge pattern may comprise one additional responsibility of a command neuron such as the MGF interneuron which organizes a rapid and powerful escape response.

Supported by a General Research Fund grant from the University of South Dakota.

513.19

ACTIVITY OF MOTONEURONS DURING ASCARIS LOCOMOTION. JA Meade and AOW Stretton.Neuroscience Training Program, U. of Wis. Madison, Wi. 53706

A semi-intact preparation has been developed in which intracellular recordings can be made from motoneurons (MN) while locomotory movements are in progress. Four classes of MNs have been investigated: VI (ventral inhibitor), DI (dorsal inhibitor), DE1 (dorsal excitor, type 1), and DE2 (dorsal excitor, type 2). The electrical activity in these neurons consists of slow potentials in VI and DI MNs, ipsps in DE1 and DI MNs, and epsps in DE2 MNs. These activities occur in bouts: DE1 ipsps and VI slow potentials are in antiphase, and DI slow potentials are in antiphase with both the DI ipsps and DE2 epsps. Simultaneous epsps in DE2 and ipsps in DI coincide with dorsal muscle contraction. The absence of psps in DE2 and DI and the presence of slow potentials in DI coincide with dorsal muscle relaxation. Based on these data we suspect that the bouts of neural activity may control the behavioral activity. If true, manipulation of the membrane potential of the appropriate MNs should influence the generation of the wave forms. To test this, we simultaneously depolarized DE2 and hyperpolarized DI, producing a dorsal flexion. When the polarity of each stimulus was reversed, a ventral flexion was observed. These results show that the nervous system alone can control the posture of the worm. Whether the nervous system is solely responsible for controlling the propagation of the waveform remains to be determined.

513.20

EXCITATION-CONTRACTION COUPLING IN A PROTOZOAN. **David C. Wood. Department of Behavioral Neuroscience, University of Pittsburgh, Pittsburgh, PA 15260.**

The trumpet-shaped protozoan, Stentor coeruleus, contracts in 10 msec after the initiation of an action potential. The Ca^{++} channels which produce these action potentials are located in the ciliary plasma membrane, whereas the contractile fiber system, the "M" bands, lies 0.5 to 1 um beneath the ciliary membrane and does not contact it directly. Due to the small magnitude of the inward Ca^{++} current and the distance of the "M" bands from the ciliary membrane, action potentials in the cilia result in too little Ca^{++} to produce contractions. This conclusion is reinforced by the observation that occasionally Stentor produce action potentials but do not contract.

Microelectrode recordings indicate Stentor contain an intracellular compartment which is surrounded by excitable membrane and is instrumental in producing contractions. With a current electrode in this compartment and a recording electrode in the cytoplasm, inward (hyperpolarizing) current pulses elicit 2-20 mV depolarizations which trigger contractions. Occasionally these intracellular depolarizations elicit ciliary action potentials. With both electrodes in the compartment inward current pulses elicit negative-going spikes which also trigger contractions. Assuming this intracellular compartment includes the large vacuoles which nearly surround the "M" bands and also appose the plasma membrane, electrical models and computer simulations which quantitatively describe the microelectrode recordings were developed. According to these models action potentials in the ciliary membrane trigger action potentials in the vacuolar membrane causing the release of Ca^{++} into the "M" bands to produce bodily contraction.

ION CHANNEL MODULATION AND REGULATION II

514.1

DEPHOSPHORYLATION OF RAT BRAIN SODIUM CHANNELS BY PURIFIED PHOSPHOPROTEIN PHOSPHATASES. S. Rossie* and W.A. Catterall. (SPON: L. Halpern). Department of Pharmacology, University of Washington, Seattle, WA 98195.

The voltage-sensitive sodium channel purified from rat brain consists of 3 subunits, α (260 kDa), β1 (36 kDa) and β2 (33 kDa). The channel-forming α subunit is phosphorylated by cAMP-dependent protein kinase *in vitro* and *in situ* at 4 or 5 cAMP-dependent phosphorylation sites clustered in a single cytoplasmic loop of the channel protein. In order to understand more completely how the steady state level of channel phosphorylation is regulated, we examined the ability of purified phosphoprotein phosphatases to dephosphorylate sodium channels.

Purified sodium channels were phosphorylated with γ^{32}P-ATP to the extent of 2-5 mol P per mol channel using the catalytic subunit of cAMP-dependent protein kinase. The phosphorylated channels were incubated with protein phosphatase, and the release of ^{32}P measured. The calcium and calmodulin dependent phosphatase calcineurin, purified from bovine brain, rapidly dephosphorylated soluble or reconstituted sodium channels, releasing 40% of the channel-associated ^{32}P within 30 min at 30°C. Phosphatase 2A, purified from rabbit skeletal muscle, dephosphorylated sodium channels less rapidly than calcineurin, but reached a similar extent after 30 min. Phosphatase 1, purified from rabbit skeletal muscle, dephosphorylated both soluble and reconstituted sodium channels to a limited extent, removing less than 10% of the channel-associated ^{32}P after 30 min at 30°C. We are currently examining whether these phosphatases are selective in their site(s) of dephosphorylation.

514.2

cAMP ACTIVATES SLOW Na^+ CURRENT WITHOUT PHOSPHORYLATION. Huang, R.-C. and Gillette, R. Dept. Physiol. & Biophys., University of Illinois, Urbana, IL 61801.

The role of phosphorylation in activation of cAMP-dependent Na^+ current ($I_{Na,cAMP}$) of molluskan neurons has been uncertain. We tested the dependence of $I_{Na,cAMP}$ on phosphorylation in neurons of the seaslug Pleurobranchaea in three ways:

1) Hill plots of injected cAMP/$I_{Na,cAMP}$ amplitude yielded a slope of nearly 1.0. The implied non-cooperativity is uncharacteristic of the conventional A-type kinase. 2) Intracellular pressure injections of the hyper-potent peptide fragment of the specific inhibitor of Kinase A (225 μM) and bath application of the kinase inhibitor H-7 (100 μM) had no significant effect on $I_{Na,cAMP}$ activation. 3) Finally, excision of the inside-out patch into normal saline silenced $I_{Na,cAMP}$ channels. Channel activity could be restored by addition of 60-1000 μM cAMP to the inside membrane face.

These data do not support a phosphorylation mechanism. Instead, they suggest that cAMP activates the $I_{Na,cAMP}$ channel through direct 1-to-1 binding at a regulatory site.

514.3

ATP AND ADENOSINE MODULATE CHANNEL ACTIVITY OF THE ACETYLCHOLINE RECEPTOR AT THE NEUROMUSCULAR JUNCTION. Z. Lu* and D.O. Smith (SPON: J. Dahl). Department of Physiology, University of Wisconsin, Madison, WI 53706.

ATP and ACh are jointly released from motor nerve terminals. ATP is then hydrolyzed to adenosine. ATP and adenosine interaction with the ACh receptor was therefore studied in dissociated adult rat muscle cells using cell-attached patch-clamp recording techniques. In the absence of ligand, spontaneous openings occurred <1/sec. With 10-uM ATP in the pipette, this rate increased to 6/sec. However, even low (0.1 uM) concentrations of ACh in the pipette increased channel opening activity to much higher levels. Although ATP may facilitate ACh channel opening, it may not be a full agonist for the ACh receptor. In contrast, with micromolar concentrations of adenosine in the pipette, channel-opening probability induced by 0.1 uM ACh was decreased by at least 30% compared to values obtained with ACh alone. Therefore, in addition to its inhibition of presynaptic ACh release, adenosine may also modulate ACh-receptor channel activity. Preliminary experiments indicate that a second messenger may be involved. Supported by NIH grant NS13500 and the M.D.A.

514.4

W7, A CALMODULIN ANTAGONIST, REDUCES NEUROMUSCULAR TRANSMISSION BY BLOCKING THE ENDPLATE CHANNEL. R.C. Marcus*, S.R. Barry. Dept. of Phys. Med. & Rehab., Univ. of Michigan, Ann Arbor, MI 48109-0042

Using intracellular recording techniques, we examined the effects of W7 [N-(6 aminohexyl)-5-chloro-1-naphthalene sulphonamide], a calmodulin antagonist, on transmission at the sartorius neuromuscular junction of the frog, Rana pipiens. W7 (5-10 μM) irreversibly reduced the amplitude of evoked endplate potentials and spontaneous miniature endplate potentials (MEPPs) but produced no changes in MEPP frequency. Thus, W7 may depress quantal size but have no effect on spontaneous transmitter release. The effects of W7 on evoked release have not yet been tested.

W7 may depress neuromuscular transmission by a mechanism independent of its effects on calmodulin. W7 is structurally similar to quinacrine, an endplate channel blocker. To test for effects of W7 on the endplate channel, we examined the effects of W7 on the rate of decay of spontaneous miniature endplate currents (MEPCs) using focal extracellular electrodes. MEPCs decreased in amplitude following application of 5-8 μM W7. In normal Ringer's, MEPCs decayed along a single exponential with a time constant of 1.2 to 5.7 msec. In the presence of W7, the time constant decreased by an average of 34%. A second, slower component of decay may have been present in W7 but was not clearly detected above the baseline noise.

These data suggest that W7 reduces neuromuscular transmission in part by blockade of the open endplate channel as opposed to an effect on calmodulin. The decrease in MEPC amplitude produced by W7 indicates that the drug may have additional postsynaptic effects.

514.5

CELL SURFACE ACH RECEPTOR EXPRESSION IN MOUSE FIBROBLASTS IS INCREASED BY cAMP-DEPENDENT KINASE STIMULATION. WN Green,* AF Ross, and T Claudio. Dept Cell & Molec Physiol, Yale Univ, New Haven, CT 06510

Different stimulators of cAMP-dependent protein kinase (PKA) were used to investigate the regulation of expresssion of *Torpedo* nicotinic acetylcholine receptors (AChRs) stably expressed in mouse fibroblast cell lines [Claudio et al. (1987) *Science* **238**:1688]. As assayed by [^{125}I]α-bungarotoxin binding, an increase (1.5 - 3-fold) in surface AChRs was detected after treatment with 3 different PKA stimulators: forskolin (10-100 mM), chlorophenylthiol (CPT) cAMP (0.5-1.0 mM), and cholera toxin (10-100 nM). Forskolin treatment resulted in a 2 - 3-fold increase in surface AChRs with a proportional increase in internal AChR complexes. Surface-expressed AChRs were equally stable in treated and untreated cells, however, differences were observed in the rate of subunit synthesis and in subunit stability. The rate of synthesis was increased 1.5-fold for α, β, γ and 2.5-fold for δ. Subunit halflives were increased 2-fold for α, β, γ and 3-fold for δ. CPTcAMP treatment resulted in a 1.6-fold increase in the number of surface AChRs, a proportional increase in internal AChRs, and no change in the stability of surface AChRs. The rate of synthesis of only the δ subunit was altered (2-fold increase) but the halflives of all four subunits were increased 2-fold. Thus, forskolin appears to increase surface expression of AChRs by increasing the rate of subunit synthesis and increasing subunit stability while CPTcAMP appears to only increase subunit stability. These results suggest that stimulation of surface AChR expression by PKA is regulated, at least in part, by posttranslational mechanisms.

514.6

Ca^{2+} ACTIVATED DEPHOSPHORYLATION DESTABILIZES $GABA_A$ RECEPTOR FUNCTION IN HIPPOCAMPAL NEURONS. Qiang X. Chen* and Robert K.S. Wong. (SPON: Stephen Gobel) Dept. of Neurology, Center for Neurobiology and Behavior, Columbia University, 630 W 168th St. N.Y., N.Y. 10032

Recent data suggest that a phosphorylation process is involved in maintaining the function of the $GABA_A$ receptor (Stelzer A.S., Kay A.R. and Wong R.K.S. 1988). We have now applied an intracellular perfusion system to further characterize the intracellular modulation sites for the receptor. Perfusion was performed during whole-cell voltage-clamp recording on acutely dissociated hippocampal neurons. Adult guinea pigs were used for the experiments. As previously reported, GABA-induced outward currents were activated and maintained when ATP (2mM), Mg2+ (4mM) and BAPTA (10 mM) were present in the recording pipette (stable solution). Addition of alkaline phosphatase (100 ug/ml) to the stable solution induced run down of the GABAA response. In addition we observed that stable responses were also obtained when ATP was substituted by ATPγ-S. GABA responses stabilized under this condition were more resistant to the phosphatase action.

Elevation of Ca^{2+} in the stable intracellular solution also caused reversible, rapid run down of the $GABA_A$ response. The run down in high Ca^{2+} was significantly retarded when GABA responses were first stabilized in ATPγ-S containing solution. In addition W7, a specific blocker of calmodulin also slowed the Ca^{2+} induced run down. Our results are consistent with the suggestion that elevation of intracellular Ca^{2+} destabilized the function of the $GABA_A$ receptor by stimulating the dephosphorylation process.

514.7

FORSKOLIN DEPRESSES CAN CURRENT IN SNAIL NEURONS. L. Donald Partridge. Dept. of Physiology, Univ. of New Mexico, Albuquerque, NM 87131.

An important role for intracellular Ca^{++} as a second messenger is in the activation of a class of calcium-activated non-specific cation (CAN) channels. These channels are found in a wide range of cell types where they either maintain depolarization or evoke secretion. (Partridge, L.D., Swandulla, D.,*TINS* 11:69-72) In snail neurons, CAN current is responsible for the pacemaker depolarizations that underlie bursting. In addition to their direct activation by Ca^{++}, these channels appear to be indirectly modulated by cyclic nucleotides.

Large neurons, most of which generated spontaneous bursts, were studied in isolated circumesophageal ganglia of *Helix aspersa*. Neurons were voltage clamped using a standard two-electrode clamp and impaled with a third electrode (100 mM KCl, 100 mM $CaCl_2$, 10 mM TRIS pH 7.5) for pressure injection of Ca^{++}. Pressure pulses were computer controlled by pCLAMP software (Axon Instruments) that also measured the resultant CAN currents. The solution bathing a neuron could be rapidly changed by switching the flow from a 500μm suprafusion pipette placed approximately 200μm from the cell.

Forskolin was used to assess the role of adenylate cyclase in the modulation of CAN currents. Application of 10μM forskolin (Sigma) rapidly and reversibly reduced CAN current (measured as total current area in nA x msec) by about 50%. Since serotonin appears to have the same effect on CAN currents as does forskolin, a mechanism involving G-proteins is implicated in the modulation of these currents. CAN currents in snail neurons have not been shown previously to be subject to modulation.

514.8

INTRACELLULAR INJECTION OF INOSITOL HEXAKISPHOSPHATE INDUCES A BIPHASIC CURRENT IN IDENTIFIED NEURONS OF APLYSIA. M. Sawada, M. Ichinose* and T. Maeno*. Dept. Physiol., Shimane Medical Univ., Izumo 693, JAPAN.

The ionic mechanism of the effect of intracellularly injected inositol hexakisphosphate (IP_6) on the membrane of identified neurons (R9-R12) of Aplysia was investigated with voltage-clamp, pressure-injection, and ion substitution techniques. Injection of IP_6 into a neuron voltage-clamped at -45 mV produced a biphasic membrane current consisting of the first inward current($I_i(IP_6)$), followed by an outward current ($I_o(IP_6)$) associated with increases in conductance at the peak of these currents. Control injections of myo-inositol were without effects. $I_i(IP_6)$ was decreased by depolarization and increased by hyperpolarization. The reversal potential of $I_i(IP_6)$ and $I_o(IP_6)$ were -34 mV and -75 mV, respectively. $I_i(IP_6)$ was sensitive to changes in $[Na^+]_o$ and $[Ca^{2+}]_o$. $I_o(IP_6)$ depended on $[K^+]_o$ and $[Ca^{2+}]_o$, and was blocked by TEA. Neither $I_i(IP_6)$ nor $I_o(IP_6)$ were sensitive to TTX. Furthermore, neither $I_i(IP_6)$ nor $I_o(IP_6)$ were blocked by 4-AP and perfusion with Cl^--deficient seawater. $I_o(IP_6)$ was completely abolished in Ca^{2+}-free plus 2 mM EGTA seawater but $I_i(IP_6)$ was partially reduced in this solution. The ionic mechanisms characterized here can lead to potent modulation of neurosecretory neurons (R9-R12) of Aplysia by IP_6.

514.9

PHORBOL ESTER INDUCES MODIFICATION OF Na-DEPENDENT ACTION POTENTIAL IN RAT SCIATIC NERVE. H. Meiri[1] and [2]B. Gross, Dep. of Physiol.&Biophys., Faculty of Med. & The Rappaport Institute, Technion, Haifa 31096, and [2]Dep. Neurol., Lady Davis Carmel Hospital, Haifa, Israel.

In rat sciatic nerve the active phorbol-1,2-beta-myristate-13-acetate (b-PMA), but not the inactive analogue 4-alpha-phorbol-12,13-didicanoate (a-PDD) induced a decrease of the action potential amplitude, its rate of rise and conduction velocity, concomitant with an increase of the threshold for action potential generation and of the duration of its refractory period. The K_d was 250nM. Exposure to b-PMA decreased TTX sensitivity from a K_d=45 nM to 400nM. The sensitivity to ATX or to Na-channel specific antibodies was not changed. The action potential completely disappeared after replacing Na with Choline. However, b-PMA had larger effects when Li replaced Na. The effects developed in the presence of Ca but were larger when Mg (but not Ba) replaced Ca. TEA or K replacement with Cs had no effect. All data implied that b-PMA works to modify Na channels.

(Supported by the Henry Gutwirth Fund for promotion of research - #184-0093)

514.10

ENDOGENOUS NEUROPEPTIDES MODULATE EXCITABILITY OF LEECH RETZIUS CELLS A.L. Kleinhaus*& C. Sahley[1] Depts. of Neurol. Sch. of Med.* & Bio.[1], Yale Univ., New Haven, CT. 06510.

The serotonergic Retzius (R) cell is a multifunction neuron implicated in the control of essential behaviors in the leech (swimming and feeding; Leake,Comp.Biochem.Phys., 1986). Last year, we reported that serotonin (5-HT) inhibited the firing rate and shortened the action potential of the R cell. We now report that in contrast to the inhibitory effect of 5-HT, SCP_b (5-10μM) and FMRF-amide (10-50μM) produce a transient depolarization and enhanced firing rate accompanied by an increase in input resistance in both *Macrobdella* and *Hirudo*. In addition, FRMF-amide alters the firing pattern from single spikes to rhythmic bursts of action potentials superimposed on slowly depolarizing waveforms followed by interburst afterhyperpolarizations. The bursting appears to be intrinsic since intercalated depolarizations and hyperpolarizations reset the burst rhythm. Under voltage-clamp, V_h between -30 mV - -80 mV, FMRF-amide flattened the steady-state I/V curve in the linear region of the curve. In the presence of TEA, FMRF-amide (50 μM) prolonged the Ca^{2+}-dependent plateau of the evoked action potential to 151 +/- 18% of the control value ($p<0.05$).

The differential modulation of excitability of the R cell by endogenous neurotransmitters might contribute to the typical firing patterns observed during various behaviors.

514.11

THE EFFECTS OF QUININE AND QUINIDINE ON ISOLATED HORIZONTAL CELLS OF THE SKATE RETINA. R. P. Malchow, H. Qian*, and H. Ripps, Depts. Ophthalmology and Anatomy & Cell Biology, Univ. Illinois College of Medicine, Chicago, Il. 60612.

Quinine and its sterioisomer quinidine are potent inhibitors of several voltage-sensitive conductances, block calcium-dependent potassium conductances, and also inhibit the activity of some membrane pumps. We report here a novel action of these agents observed in recordings from isolated retinal horizontal cells which does not fit this pattern of inhibition.

Skate retinal horizontal cells were voltage clamped using the whole-cell configuration of the patch clamp technique, and drugs applied by superfusion. 2 min applications of 100 uM quinine or quinidine markedly reduced the transient potassium current (the A current) and completely abolished the transient fast inward current carried by sodium normally observed in these cells. However, when cells were depolarized beyond 0 mV for several seconds, these drugs produced a large outward current that was accompanied by a significant increase in membrane conductance. Analysis of tail currents suggest that this current has a reversal potential near 0 mV. Both the reduction of the voltage-activated currents and the development of the slow outward current were readily reversible upon superfusion with normal Ringer. Our results suggest that the actions of quinine and quinidine on neuronal membranes are more complex than previously believed.

Supported by a research grant (EY-06516) from the N.E.I., and unrestricted awards to HR from Research to Prevent Blindness, Inc.

514.12

PHENYTOIN, BACLOFEN, TIZANIDINE AND MEMANTINE REDUCE HYPEREXCITABILITY OF NEURONS IN CULTURE BY INTERFERING WITH DIFFERENT CURRENTS. R. Netzer*, T. Binscheck*, H. Bigalke* (SPON: European Neuroscience Association). Department of Pharmacology and Toxicology, Medical School of Hannover, 3000 Hannover 61, West Germany.

The antiepileptic and anticonvulsant agents phenytoin, baclofen , memantine, and tizanidine have been examined in cultured spinal cord neurons of fetal mice. Effects of these substances on hyperactivity induced by tetanus toxin, strychnine and picrotoxin and on membrane current were compared.

Spontaneous activity of neurons was recorded by intracellular microelectrodes (70 - 100 MΩ). Application of strychnine, tetanus toxin and picrotoxin induced a hyperactivity of the neurons by blocking inhibitory synaptic transmission within the neuronal network, which is expressed by the appearance of bursts (Bergey et al, J. of Neurophysiol., 57: 121, 1987). Phenytoin, memantine, and tizanidine decreased the duration of bursts and the frequency of action potentials (AP) within the bursts, depending on the applied concentration. In contrast, baclofen decreased the frequency of bursts while leaving their duration and the frequency of AP within the bursts unaffected.

Inward and outward currents were recorded by voltage clamp technique in the 'Whole Cell' mode. Similar to phenytoin, memantine and tizanidine decreased the peak inward current over the voltage range from −120 mV to +100 mV. In contrast, baclofen decreased the peak inward current only in the voltage range from −20 mV to +100 mV. The currents were restored by switching to drug-free superfusion solution. It is concluded that the decrease of the current induced by baclofen reflects an activation of a potassium outward current, whereas the other agents directly block the sodium inward current. In addition, voltage dependent inactivation of the sodium inward current was shifted towards more negative potentials, thus leading to an earlier inactivation of inward current in the course of an AP and to an delay on its axonal propagation.

514.13

A NOVEL NEUROACTIVE PEPTIDE FROM LYCOSID SPIDER VENOM. K.Kobayashi*, S.J.Bryant*, C.R.Bryant*, L.D.Margolin, J.B.Fischer, R.N.McBurney*, A.C.Server. CNS Research, Cambridge, MA 02139. C.Miller*. Brandeis University, Waltham, MA 02254. G.R.Strichartz. Harvard Medical School, Boston, MA 02115. B.L.Roach*, J.Meinwald*. Cornell University, Ithaca NY 14853.

A novel neuroactive peptide which blocks the rat spinal reflex was isolated and purified from Lycosid spider venom using reversed-phase HPLC. The structure was determined by amino acid analysis, automated Edman degradation, and mass spectroscopy, and its activity was confirmed with chemically-synthesized peptide consisting of 23 amino acid residues. The peptide completely blocked the spinal reflex in hemisected new-born rat spinal cord at a concentration of 10 μM. It also inhibited the generation/propagation of action potential in rat phrenic nerve. By using the frog sciatic nerve with sucrose gap recording, the peptide was shown to cause TTX-insensitive membrane depolarization. The effects were irreversible. When the peptide was added to a planar lipid bilayer membrane, voltage-dependent channel activity was observed. These results indicate that the newly isolated peptide is a pore-former. The voltage dependence of the pore-forming activity is probably due to its basicity. The peptide seems to share a common structural feature with other pore-forming peptides such as mastroparan. (We thank Drs. C.E.Jahr and T.M.Jessell for their valuable advice.)

514.14

GAP JUNCTION CHANNELS ARE CALCIUM DEPENDENT, MODULATED BY PROTEIN PHOSPHORYLATION AND pH. R. O. Arellano, F. Ramón and A. Rivera. Depto. de Fisiología. CINVESTAV. Apdo. Postal 14-740. México, MEX. 07000.

Lateral axons from the crayfish nerve cord are segmented and coupled by gap junctions. We examined the coupling resistance (Rj) after internal perfusion with solutions containing different calcium concentrations at pH 6.0 and 7.0, and in the presence or absence of a phosphorylating cocktail.

The external solution was van Harreveld's. The Standard Internal Solution (SIS) contained (in mM): NaCl 15, KF 33, K-glutamate 187, sucrose 12, and MOPS or MES 15, for pH 7 or 6. The phosphorylating cocktail (SIS-P) contained SIS and (in mM): MgCl2 4, ATP 5, cAMP 0.01, and cAMP dependent protein kinase (PK) 100 µgr/ml. Calcium in internal solutions was measured with calcium sensitive electrodes and buffered with EGTA (2-5 mM). Solutions with pCa >7 had 10 mM EGTA or 10 mM BAPTA for pH 7 or 6.

Results include: 1) in the absence of a phosphorylating cocktail high calcium or low pH solutions had no effect on Rj over 20 min perfusion; 2) SIS-P at pH 7 induced a calcium dependent increase in Rj; the dose-response curve had a Km of 1 µM calcium; 3) SIS at pH 6 induced a calcium dependent increase in Rj with a Km of 1 µM; 4) SIS-P at pH 6 increased Rj with a Km of 0.1 µM calcium. Phosphorylation was assessed by: a) perfusion with SIS-pCa 5 containing AMP-PCP or Welsh inhibiton, which had no effect on Rj, and; b) perfusion of the axons with SIS-pCa 5 and the catalytic subunit of the cAMP dependent PK, which had an effect on Rj similar to that observed with SIS-P. We conclude that gap junctions from crayfish lateral axons are closed by direct binding of calcium ions to a site that is exposed after protein phosphorylation or protonation. Of these, phosphorylation seems a more physiological mechanism. (SPON: G. Escalona de Motta)

514.15

PARTICIPATION OF CAFFEINE- AND RYANODINE-SENSITIVE Ca^{++}-STORES IN LOW pH_i-INDUCED UNCOUPLING OF CRAYFISH AXONS C. Peracchia. (SPON: R.A. Eatock). Dept. Physiology, Univ. Rochester, Rochester, NY 14642

We have previously shown with ion selective microelectrodes that, in low pH_i-induced gap junction uncoupling of crayfish septate axons, junctional resistance (R_j) and $[Ca^{++}]_i$ curves match well with each other while R_j and $[H^+]_i$ curves do not match. This suggested a Ca^{++}-mediated gating mechanism (Peracchia, C., *Biophys. J.*, 55:151a, 1989). To investigate the mechanism of $[Ca^{++}]_i$ increase we have studied the effects of different $[Ca^{++}]_o$, Ca-channel blockers and drugs that affect Ca-release from stores. Neither changes in $[Ca^{++}]_o$ nor Ca-channel blockers had any effect. In contrast, caffeine affected both R_j and $[Ca^{++}]_i$ maxima reached with acetate(Ac)-induced low pH_i. Superfusion with Ac-salines containing 10 mM caffeine increased substantially the Rj maxima, while exposure to caffeine or ryanodine (1 μm) before, during and after Ac superfusion reduced significantly the R_j maxima with Ac. Preliminary experiments with Ca-microelectrodes show that, with caffeine, $[Ca^{++}]_i$ curves match well with R_j curves. These data indicate that cytoplasmic acidification closes channel gates via Ca^{++} released from internal stores. Vesicles (70 nm) coating both junctional surfaces could be Ca^{++}-storing organelles providing the means for both Ca^{++}-buffering and Ca^{++}-release. (Peracchia, C. and Dulhunty, A. *J. Cell Biol.*, 70:419, 1976). Supp. by NIH Gr. GM20113.

MUSCARINIC RECEPTORS

515.1

BINDING CHARACTERISTICS OF ^{3}H-4DAMP, A PUTATIVE MUSCARINIC-M3 ANTAGONIST, IN RAT BRAIN. R. Quirion, D. M. Araujo, & P. A. Lapchak. Douglas Hosp. Res. Ctr., McGill Univ., Montreal, Quebec, Canada H4H 1R3.

Based upon results from competitive inhibition studies and functional tests, 4DAMP was originally classified as a selective muscarinic-M3 antagonist. Using membrane binding and autoradiographic techniques, we attempted to characterize the binding of ^{3}H-4DAMP in the rat brain. In homogenates of tissue from various brain regions, ^{3}H-4DAMP appears to bind two classes of muscarinic sites, one with high affinity (Kd=0.14-0.53 nM) and low capacity (Bmax=19-122 fmol/mg protein) and a second with lower affinity (Kd=49-99 nM), higher capacity (Bmax=141-860 fmol/mg protein). Competitive inhibition experiments using either 0.5 or 50 nM ^{3}H-4DAMP showed a different ligand selectivity pattern for the two classes of sites, with pirenzepine exhibiting greater potency at the lower affinity sites. Moreover, autoradiographic analysis of ^{3}H-4DAMP binding reveals dense labeling in the deep and superficial cortical layers, the hippocampal CA1 and dentate gyrus regions, and the external plexiform layer of the olfactory bulb, a pattern somewhat reminiscent of muscarinic-M1 binding in these areas. However, moderate to dense labeling was also observed in other brain structures, such as brainstem nuclei, which are not enriched with muscarinic-M1 sites. In summary, it appears that ^{3}H-4DAMP may be a useful ligand to study both the M1 and the M3 subtype of muscarinic receptor.

515.2

REGIONAL DIFFERENCES IN THE BINDING OF 4-DAMP TO RAT BRAIN: COMPARISON WITH MINIMUM ENERGY CONFORMATIONS. D.A. Collins*, D.A. Smith* and W.S. Messer, Jr. (SPON: C.L. Hinman) Dept. of Medicinal and Biological Chemistry, College of Pharmacy and Dept. of Chemistry, Univ. of Toledo, 2801 W. Bancroft St., Toledo, OH 43606

The binding of the muscarinic antagonist 4-DAMP, which is M_3-selective in the periphery, was examined through quantitative autoradiographic techniques in brain. The ability of 4-DAMP to displace [^{3}H]-l-quinuclidinyl benzilate (QNB) binding to rat brain sections was compared with the known distribution of M_1 and M_2 muscarinic receptor subtypes as measured previously with pirenzepine and AF-DX 116. 4-DAMP displayed a high affinity for [^{3}H]-l-QNB binding sites in rat brain sections. Analysis of 4-DAMP binding to various regions of rat brain revealed heterogeneous binding profiles, suggesting an interaction with multiple receptor sites.

Quantification of the autoradiograms indicated that 4-DAMP bound with the highest affinity to muscarinic receptors in the midline thalamus (IC_{50} values < 30 nM), and a slighlly lower affinity for hippocampal receptors (IC_{50} values between 30 and 46 nM). 4-DAMP also displayed a lower affinity for cortical receptors with IC_{50} values between 30 and 50 nM. The binding profile of the putative M_3 muscarinic antagonist did not exhibit a marked selectivity for any single region of brain. The data suggest that whereas 4-DAMP is selective for M_3 receptors in the periphery, it has only marginal selectivity in the CNS. The design and synthesis of muscarinic antagonists with M_3-selectivity in the CNS is an important goal for future work.

Minimum energy conformations for 4-DAMP were calculated using the MM2 force field implemented on C. Still's program MacroModel (version 2.0). 4-DAMP displayed two global minimum energy conformations, differing in the relative position of the piperidine ring with respect to the aromatic rings. The minimum energy conformations of 4-DAMP were compared with the conformations generated for pirenzepine (Messer et al., J. Med. Chem. in press). The lowest energy conformation of 4-DAMP was superimposable on the lowest energy conformation of pirenzepine (RMS = 0.297 Å). It is suggested that the conformations available to 4-DAMP allow it to bind to all muscarinic receptors in the CNS. Supported by NS 23929 and NS 25765.

515.3

RADIOLIGAND BINDING CHARACTERIZATION OF MUSCARINIC RECEPTOR SUBTYPES IN CEREBRAL BLOOD VESSELS. A.L. García-Villalón*, D. Krause and S.P. Duckles. Dept. of Pharmacology, School of Medicine, Univ. of California, Irvine. 92714 CA.

Muscarinic cholinergic receptor sites in bovine cerebral arteries were analyzed by using radioligand binding assays with the specific muscarinic antagonist [^{3}H]-quinuclidinyl benzilate ([^{3}H]-QNB) as ligand. Specific binding of [^{3}H]-QNB to membrane preparations from pial arteries was saturable, of high affinity (K_D = 6.8 ± 1.6 x 10^{-10} M) and inhibited by the muscarinic antagonists atropine, 4-diphenylacetoxy-N-methylpiperidine metho-bromide (4-DAMP), dicyclomine, 11-2[[2-(diethylamino) methyl]-1-piperidinyl]acetyl]-5,11-dihydro-6H-pyrido[2,3-b][1,4]benzodiazepin-6-one (AF-DX 116) and pirenzepine. The order of potency for the antagonists was atropine (pK_i=8.43) > 4-DAMP (pK_i=7.66) > dicyclomine (pK_i=7.00) > AF-DX 116 (pK_i=6.75) > pirenzepine (pK_i=6.15), and the Hill coefficients were not significantly different from 1. These results suggest that only one class of muscarinic binding site is present in bovine cerebral arteries and that this site is of the M_2 type. These findings contrast with previous studies in this laboratory with bovine coronary arteries and rabbit ear arteries that suggest these peripheral vascular receptors are of the M_3 type. *(Fogarty Fellowship #1F05 TWO 4078 and N.I.H. grant #DK36289).*

515.4

MUSCARINIC RELAXATION OF THE CAT MIDDLE CEREBRAL ARTERY IS MEDIATED BY M3 RECEPTORS. F. Dauphin* and E. Hamel. Laboratory of Cerebrovascular Research, Montreal Neurological Institute, Montreal, Quebec, H3A 2B4.

The subtype of muscarinic receptor involved in the endothelium-dependent relaxation to acetylcholine (ACh) was pharmacologically characterized in the cat middle cerebral artery. Cholinergic agonists such as carbachol, ACh and methacholine elicited maximal relaxations (E_{Am}) which corresponded to 75-96% of the induced tone. Oxotremorine and the M_1 agonist McN-A-343 were significantly less potent vasodilators (E_{Am} of 51% and 24% of the tone, respectively). However, affinities of most agonists at the cerebrovascular receptor were comparable (pD_2 values ranging from 7.15 to 7.60) except for McN-A-343 and carbachol which exhibited respectively higher and lower affinity. Non-selective muscarinic antagonists (atropine, scopolamine and quinuclidinyl benzilate (QNB)) were the most potent inhibitors of the ACh-induced relaxation. Selective antagonists at the M_1 (CNS), M_2 (cardiac) and M_3 (glandular) subtypes of pharmacological muscarinic receptors were found to potently and competitively inhibit the vasomotor response to ACh. The order of potency of various antagonists can be summarized as: QNB ≥ scopolamine > atropine ≥ 4-DAMP > hexahydrosiladifenidol (HHSiD) ≥ dicyclomine > pirenzepine ≥ adiphenine > methoctramine ≥ AF-DX 116 > gallamine. The pA_2 values for these antagonists varied from 10.87 ± 0.09 (QNB) to 5.29 ± 0.09 (gallamine). The pharmacological features of the cerebrovascular muscarinic receptor: low efficacy of M_1 agonist (McN-A-343), intermediate affinity for M_1 antagonists such as pirenzepine, low affinity for M_2 selective drugs (AF-DX 116 and methoctramine) and high affinity for M_3 antagonists (4-DAMP, HHSiD) strongly suggest that a site similar to the M_3 subtype of muscarinic receptor is mediating the cholinergic relaxation in the cat cerebrovascular bed. Supported by the MRC of Canada (EH) and a Jeanne Timmins Fellowship (FD).

515.5

EVIDENCE OF THE M3 MUSCARINIC RECEPTOR SUBTYPE IN HIPPOCAMPAL NEURONS. T.A. Pitler and B.E. Alger. Dept. Physiol., Univ. MD. Sch. Med., Baltimore, MD 21201.

Recent pharmacological and molecular evidence indicates that up to four distinct muscarinic receptor subtypes may exist in the brain. Pharmacologically, these receptors can be defined by their affinities for certain muscarinic antagonists. The various actions of muscarinic agonists in hippocampal neurons may be mediated by multiple receptor subtypes, although there is little quantitative data on this point. To test the hypothesis we used Schild plot analysis to determine the affinities of muscarinic antagonists pirenzepine, 4-DAMP and AFDX-116 for blocking muscarinic responses. We studied the carbachol-induced input resistance increase in hippocampal neurons with intracellular recording in the rat hippocampal slice.

Following determination of a limited dose-response curve for carbachol in a cell, alternating doses of antagonist and agonist were given to determine antagonist-induced rightward shifts in the dose-response relationship. At sufficient doses all antagonists blocked the increase in input resistance. The block was in turn overcome by higher doses of agonist. Schild analysis revealed affinites of 7 nM for 4-DAMP, 280 nM for pirenzepine and 2 uM for AFDX-116. The order of potency and quantitative values are in close agreement with the antagonist binding affinities for the M3 receptor and strongly suggest a role for this receptor subtype in brain.

515.6

CLASSIFICATION OF FUNCTIONAL M2 MUSCARINIC RECEPTORS IN THE CORTEX AND STRIATUM. D.J. Anderson, M. McKinney, C. Forray[1], and E. El-Fakahany[1]. Neuroscience Research Division, Pharmaceutical Discovery, Dept. 47W, Abbott Laboratories, Abbott Park, IL 60064.

Central M2 muscarinic receptors have been shown to be coupled to cyclic AMP inhibition which pirenzepine blocks with low affinity. Further, detailed characterization was achieved using AF-DX 116, 4-DAMP, HSD, and methoctramine (MET), which have differential selectivities for M2 receptors in various tissues. Mechanically-dissociated cellular aggregates from adult rat brain were prelabelled with [^{3}H]adenine. Carbachol response curves of the inhibition of forskolin-stimulated [^{3}H]cyclic AMP accumulation were shifted with various concentrations of muscarinic antagonists. K_d values were determined from "Schild" analysis.

The striatal M2R displayed K_d values of 155 nM for AFDX, 0.2 nM for 4-DAMP, 14 nM for HSD, and 47 nM for MET. Similar values for AFDX and 4-DAMP were determined in the cortical M2R. However, differences between cortex and striatum were observed with MET and HSD. These data indicate that the M2R in both the striatum and cortex are non-cardiac.

515.7

THE BINDING PHARMACOLOGY OF VARIOUS MUSCARINIC RECEPTOR ANTAGONISTS AND AGONISTS AT M1 RECEPTORS EXPRESSED IN M1C2 CELLS AND RAT BRAIN CORTICAL TISSUE. S. Myers*, L. Coughenour*, D. Dudley, J. Fergus*, S. Fisher*, L. Lauffer*, C. Clark. Pharmacology Department, Parke-Davis Pharm. Res. Div., Warner-Lambert Co., Ann Arbor, MI 48105.

The binding pharmacology of representative muscarinic receptor antagonists and agonists at cloned M1 receptors was compared with binding results using rat cerebral cortex membranes as an "M1" receptor source. Binding inhibition curves for test agents were determined against [3H]-quinuclidinyl benzilate (QNB) labeled receptors. The rank order of antagonist binding potencies did not differ between receptor sources. Antagonist affinities were about 2 fold higher at cloned M1 receptors compared to cortical receptors with two exceptions: pirenzepine and hexahydrosiladifenidol bound with 20 fold higher affinity at cloned M1 receptors compared to cortical receptors. The relative rank order of agonist binding affinities differed with receptor source. Oxotremorine-M had a 4 fold lower affinity and its potency rank order relative to the other agonists tested was right-shifted at cloned M1 receptors compared to its affinity and relative rank order potency at cortical receptors. Agonist inhibition curves had slope values near unity and were not affected by guanine nucleotides. These results extend the binding pharmacology of muscarinic antagonists and agonists to M1 receptors expressed in M1C2 cells and demonstrate that drug affinities at M1 receptors are different from those obtained using cortical tissue possibly due to the presence of multiple muscarinic subtypes. *Dr. C. Venter is acknowledged for supplying M1C2 cells.

515.8

COMPARISON OF THE BINDING PROFILE OF ALLOSTERIC ANTAGONISTS AT THE MUSCARINIC RECEPTORS OF RAT BRAIN. Norman H. Lee and Esam E. El-Fakahany. Department of Pharmacology and Toxicology, University of Maryland School of Pharmacy, Baltimore, MD 21201.

The potency of various allosteric antagonists to displace and modulate ligand binding to the muscarinic receptor (MR) was investigated in brainstem (BS), cerebellum (CB), heart (H) and cortex (CTX; pirenzepine low-affinity sites occluded with propylbenzilycholine mustard in presence of 100 nM pirenzepine). In BS, CB and H, the rank order of potency to decelerate [^{3}H]NMS dissociation kinetics was determined to be methoctramine (Met) > gallamine (Gall) > PCP $\simeq$ verapamil (Ver). The potassium channel blocker, 4-aminopyridine (4AP) was without effect. Met, Gall, PCP and Ver exhibited biphasic [^{3}H]NMS competition curves in BS, CB and CTX while 4AP bound with a single high affinity (Ki = 62 $\pm$ 6 nM in CB, mean $\pm$ S.E.M.). Met and Gall are unique because of their reputed M_2-selectivity. Indeed, Met and Gall exhibited ~10-fold greater affinity in BS, CB and H than in CTX. The degree of negative cooperativity exerted by Met, Gall, PCP and Ver in the CTX was modest in comparison to BS, CB and H. In conclusion, the affinity profile of the allosteric antagonists at the MR in BS, CB and H correlates well with their allosteric potency. Supported in part by NIH grants NS-24158, AG-07118, AG-00344.

515.9

PHARMACOLOGICAL EVIDENCE FOR THREE MUSCARINIC BINDING SITES IN RAT BRAIN. *T.S. Smith, S.J. Edmond, F.J. Ehlert and F.M. Leslie.* University of California, Irvine, CA. 92717

Recent studies by Ehlert et al. (FASEB J. 3: A1285, 1989) have indicated that [3H]N-methylscopolamine (NMS) binding to membranes of the rat corpus striatum is best explained by a model consisting of at least three populations of muscarinic binding sites. While the M1 and M2 muscarinic receptors can be labeled by pirenzepine (PZ) and AF-DX 116, respectively, these two sites only account for 37% of the total sites labeled by [3H]NMS. We have used radioligand slice binding and quantitative autoradiography to confirm the existence of the three muscarinic binding sites in the rat brain. [3H]NMS binding was examined in the presence of PZ (100nM) and AF-DX 116 (500nM) to block radioligand interaction with both the M1 and M2 receptors. Under these conditions, [3H]NMS labels a large population of binding sites. This binding can be completely displaced by 1 μM atropine, indicating that [3H]NMS is binding to a muscarinic site. The pharmacological profile of this [3H]NMS binding site, as determined from competition studies, is distinct from that of the M1 and M2 receptors. While the muscarinic ligands 4-DAMP and NMS display a high potency for inhibition of [3H]NMS binding (Ki values 9.6nM and 0.61nM, respectively), the M2 selective ligand, AF-DX 116, and the M1 selective ligand, PZ, both display relatively low affinities for the [3H]NMS binding site (Ki values 4500nM and 810nM, respectively). Differential autoradiographic distributions of [3H]NMS, [3H]AF-DX 116 and and [3H]PZ binding have provided further evidence that [3H]NMS is labeling a non-M1, non-M2 binding site. While there is overlap in the distribution of radioligand binding sites in some brain regions, other structures are densely labeled by only one radioligand. These data indicate that, under conditions where binding to the M1 and M2 receptors is blocked by selective ligands, [3H]NMS binds to a third population of non-M1, non-M2 muscarinic sites.
Supported by PPG 9-445150-3002 and PMA fellowship 9-405150-52883.

515.10

AN APPROACH TO THE DEVELOPMENT OF ANTIBODIES TO SUBTYPES OF MUSCARINIC RECEPTORS. **S.J. WALL, F. HORY*, S. FLAGG*, B.M. MARTIN*, E.I. GINNS*, AND B.B. WOLFE. Dept. of Pharmacol., Univ. of Pennsylvania Sch. Med. Philadelphia, PA, 19104, N.I.M.H., Bethesda, Md. 20892, Dept. of Pharmacol., Georgetown Univ. Sch. Med. Washington, D.C. 20007.**

A 639 base pair cDNA encoding 213 amino acids (Arg-134 to Lys-346) of the m1 muscarinic acetylcholine receptor (m1 MAChR) was isolated from a λgt11 rat brain library using a 44 base oligonucleotide probe. This cDNA fragment was subcloned into pBR322, and after digestion of the construct with Sma I, an Eco RI linker was introduced at Arg-220. Using Eco RI, a fragment encoding 127 amino acids of the third intracellular loop of the m1 MAChR was obtained and ligated into pRIT23, a vector producing fusion proteins consisting of a truncated portion of Staphylococcal protein A (SPA) and the peptide corresponding to the introduced cDNA. We plan to produce a fusion protein containing the 127 amino acid peptide of the m1 MAChR. Using the truncated SPA portion of the fusion protein which contains domains binding the Fc portion of mammalian IgG, fusion protein will be purified on IgG Sepharose and used for immunization. Antibodies produced in this manner should provide selective probes for subtypes of muscarinic receptors in tissues expressing mixed populations of MAChRs. (Supported by NS26934, GM31155 and MH14654)

515.11

CLONING, EXPRESSION, LOCALIZATION AND FUNCTIONAL EXPRESSION OF A MUSCARINIC ACETYLCHOLINE RECEPTOR FROM DROSOPHILA. R.A. Shapiro*, E.M. Subers*, B. Wakimoto* and N.M. Nathanson. Departments of Pharmacology and Zoology, University of Washington, Seattle, WA 98195

We have cloned a cDNA encoding a *Drosophila* muscarinic acetylcholine receptor, inserted it in an expression vector under the control of the zinc-inducible metallothionein promotor and expressed it in the Y1 adrenocarcinoma cell line. The cloned receptor is able to bind a variety of muscarinic specific agonists and antagonists and has a high affinity for quinuclidinyl benzilate and atropine and a low affinity for the muscarinic antagonist pirenzipine. Activation of the receptor by the agonist carbachol increases the production of total inositol phosphates and the level of cAMP accumulation in intact cells. *In situ* hybridization of the receptor gene to *Drosophila* salivary gland chromosomes localizes the gene to the distal end of the right arm of chromosome 2. Analysis of a genomic clone of the gene reveals that there is an intron located in the third cytoplasmic loop of the receptor.

515.12

ALLOSTERIC REGULATION OF CLONED MUSCARINIC RECEPTOR SUBTYPES. J. Ellis, J.H. Huyler* and M.R. Brann. Neuroscience Research Unit, Dept. of Psychiatry, Univ. of Vermont, Burlington, VT 05405 and Laboratory of Molecular Biology, NINCDS, Bethesda, MD 20892.

Membranes prepared from cells containing single subtypes of muscarinic receptors (*Buckley et al., Mol. Pharmacol. 35:469, 1989*) were labeled with [^{3}H]N-methylscopolamine (NMS) or [^{3}H]quinuclidinylbenzilate (QNB) in 5 mM Na-K phosphate, pH 7.4 (PB). Half-times of dissociation of [^{3}H]NMS (initiated by 1 μM atropine at 23°C) ranged from 5 min for the m_2 receptor to 60 min for the m_5 receptor; for [^{3}H]QNB, half-times (37°C) ranged from 1 hr (m_2) to 3 hr (m_3). The presence of gallamine slowed dissociation of [^{3}H]NMS from all of the subtypes, with an order of potency of $m_2 > m_1 \approx m_4 > m_3 \approx m_5$. Dissociation of [^{3}H]QNB from the m_1, m_2, and m_4 subtypes was modulated by gallamine in the biphasic manner described previously for cardiac receptors (*Ellis and Seidenberg, Mol. Pharmacol. 35:173, 1989*); that is, low concentrations (1-10 μM) of gallamine accelerated dissociation, while 1 mM gallamine slowed it. Dissociation of [^{3}H]QNB from the m_3 and m_5 subtypes was essentially unaffected by gallamine. The potency of gallamine in modulating the dissociation of both ligands was reduced by increasing ionic strength (to 50 mM PB). These findings indicate that susceptibility to allosteric regulation varies in a complex way across subtypes. *Supported by R01AG05214.*

515.13

SECRETED FACTORS REGULATE EXPRESSION OF AVIAN RETINA mAChR SUBTYPES IN VITRO. A.F. SKORUPA AND W.L. KLEIN. Dept. of Neurobiology and Physiology, Northwestern Univ., Evanston, IL 60208.

Muscarinic acetylcholine receptors (mAChR) exist in the chicken central nervous system in two molecular weight subtypes (86kD and 72kD), and the relative proportions of these shift during synaptogenesis in vivo. In culture, the shift occurs in high density but not low density monolayer cultures, supporting the hypothesis that cell-cell interactions are involved. Further data support a role for secreted factors. Transfer of high-density cultures, plated on coverslips, to larger plates containing a greater volume of media decreased the expression of the mature 72kD form; this expression was restored by growing the cultures in media conditioned by high density cultures (CM). Similarly, growing low density cultures in CM increased the proportion of the 72kD subtype. A striking difference in receptor differentiation was seen when the effects of CM collected from young cultures (CM1-4) was compared with that from older cultures (CM5-8). Cultures grown with CM5-8 developed a major peak of the 72 kD subtype, while those grown with CM1-4 showed very low levels. These data support a role for developmentally-regulated secreted factors in the expression of mAChR subtypes. (Supported by NIH grants NS21234, NS21088 & NS23348 to WLK.)

515.14

MUSCARINIC RECEPTOR REGULATION: REGIONAL AND SUBTYPE-SPECIFIC EFFECTS FOLLOWING CHOLINESTERASE INHIBITION. K.A. Frey, M.M. Howland* and B.W. Agranoff. M.H.R.I., Univ. of Michigan, Ann Arbor, MI 48104-1687 (USA).

Previous studies have detected alterations in regional muscarinic receptors (MAChR) following chronic administration of muscarinic agonist or antagonist agents. In the present study, we examined the possibility of selective muscarinic receptor subtype regulation in the rat following one week of acetylcholinesterase inhibition by DFP. Muscarinic receptor densities were determined with the use of [^{3}H]scopolamine. The selective antagonists pirenzepine (PZ) and AFDX116 (AFDX) were used as competing ligands to visualize remaining MAChR subtypes. Quantitative autoradiography was used to determine regional B_{max} and k_D of [^{3}H]scopolamine binding and the k_I for high and low affinity binding of both competitors. In control animals, there was regional heterogeneity in the relative proportion of low affinity PZ vs. AFDX binding sites, with maximal distinctions observed in the dentate gyrus (high proportion of M1) and the hypoglossal nucleus (high proportion of M2). In DFP treated animals, a regionally-specific pattern of receptor down-regulation was observed. Brain regions with high levels of presynaptic cholinergic input (cortex, striatum, hippocampus) showed significant reductions, while regions devoid of significant cholinergic input (dorsal thalamus, cerebellum, pons) were unaffected. Subtype analysis revealed loss of both high and low affinity sites for PZ and AFDX. In the striatum, there was greater reduction in low affinity PZ sites (51%) as compared to low affinity AFDX sites (32%). Taken together, these results suggest that the MAChR changes following cholinesterase inhibition rely on endogenous cholinergic input, and that several if not all of the subtypes participate in regulatory responses. The data from the striatum further suggest that individual subtypes of MAChR may be differentially regulated.

515.15

CELL CYCLE DEPENDENT REGULATION OF MUSCARINIC-STIMULATED NE RELEASE AND PI HYDROLYSIS IN PC12 CELLS. A. Takashima* and J. G. Kenimer. Lab. Cell. Physiology, CBER, FDA, Bethesda, MD 20892

PC12 cells, plated at 50,000 cells per well, reached stationary phase after 11 days in culture. Methacholine (300 µM)-stimulated norepinephrine (NE) release was maximal at day 2 and then rapidly decreased during subsequent cell growth to a value which was only 5 per cent of maximal. In contrast, methacholine-stimulated phosphoinositide (PI) hydrolysis increased to a maximum at day 8 and then decreased to a value of approximately 20 per cent above basal during the stationary phase. Muscarinic receptor antagonist binding was maximal at days 2 and 8 and then decreased to approximately 25 per cent of maximum at day 15. Cells were arrested in G1 phase by growth in medium without isoleucine and glutamine and the incorporation of [3H]thymidine into DNA measured after return to normal medium. [3H]thymidine uptake increased during the first 16 h and then decreased during the next 24 h. Methacholine-stimulated NE release was maximal at time 0 (G1 phase), decreased to 20 percent of maximum at 24 h and then increased to 50 percent of maximum at 40 h. Methacholine-stimulated PI hydrolysis was maximal at 24 h and then decreased to original levels over the next 16 h. Muscarinic receptor antagonist binding decreased for the first 16 h and then increased to original G1 phase levels at 40 h. These results support our hypothesis (Takashima and Kenimer-J.Biol.Chem.-In Press) that muscarinic-stimulated NE release and PI hydrolysis are independent events controlled by separate muscarinic receptor subtypes and suggest that these distinct receptors are differentially expressed during the cell cycle.

515.16

DESENSITIZATION AND SEQUESTRATION IN TWO MUSCARINIC RECEPTOR SUBTYPES. J. Baumgold and B. Cooperman. Lab. of Mol. and Cell. Neurobiology, NINDS, Bethesda, MD 20892.

Agonist-induced sequestration and desensitization of receptors was studied in two cell lines that each express a differentially coupled muscarinic receptor subtype. NG108-15 glioma x neuroblastoma cells express only m4 receptors coupled to adenylate cyclase inhibition, whereas SK-N-SH human neuroblastoma cells express only m3 receptors coupled to PI turnover. In both cell lines, muscarinic agonists caused a rapid temperature-dependent loss of cell-surface receptors. These sequestered receptors could be detected with the hydrophobic ligand [^{3}H]-quinuclidinyl benzilate ([^{3}H]-QNB) but not with the hydrophilic ligand [^{3}H]-N-methylscopolamine ([^{3}H]-NMS). Furthermore, agonist treatment of intact cells caused the receptors to re-distribute to a lighter membrane fraction after differential centrifugation of cell lysates. In contrast to this sequestration, only the response mediated by m4 receptors from NG108-15 cells became desensitized, as measured by a decrease of the appropriate second-messenger response. Therefore, although the m3 receptors in SK-N-SH cells did become sequestered in response to agonists, they did not desensitize. These findings demonstrate that agonist-induced receptor sequestration does not necessarily lead to receptor desensitization.

515.17

MUSCARINIC RECEPTOR ACTIVATION OF A PUTATIVE TRANSCRIPTION FACTOR GENE, zif/268, IN N1E-115 NEUROBLASTOMA CELLS: ROLE OF THE M2-LIKE RECEPTORS. D.W. Saffen*, E.S. Erol*, P.F. Worley and J.M. Baraban Dept. of Neuroscience, The Johns Hopkins University, Baltimore MD 21205

Recent studies suggest that cell surface receptor stimulation by neurotransmitters can elicit a rapid genomic response. To help define the mechanisms mediating this response, we have examined muscarinic receptor activation of zif/268, a putative transcription factor gene, in N1E-115 neuroblastoma cells. This cell line contains two pharmacologically-defined muscarinic receptors: "M2"-like receptors linked to reduction of cyclic AMP levels, and "M1"-like receptors linked to stimulation of cyclic GMP synthesis and PI turnover. The "M2" receptor-specific agonists oxotremorine and arecoline rapidly increase zif/268 mRNA levels in N1E-115 cells, with mRNA reaching maximal levels within one hour. The induction is blocked by 2 μM atropine, a muscarinic antagonist. Induction of zif/268 mRNA by oxotremorine (50 μM) is also blocked by pre-treatment of the cells with 5 ng/ml pertussis toxin for 18 hours. These data suggest that muscarinic receptor-mediated activation of zif/268 does not require stimulation of the cGMP and PI systems and is mediated by activation of the M2-like receptors via a pertussis toxin sensitive G protein.

515.18

COMPETITIVE AND NON-COMPETITIVE ANTAGONISM OF MUSCARINIC RECEPTOR-MEDIATED RESPONSES BY METHOCTRAMINE.
Carlos Forray, Norman H. Lee*, Michael McKinney, and Esam E. El-Fakahany. Department Pharmacology and Toxicology, University of Maryland School of Pharmacy, Baltimore, MD 21201.

In an attempt to assess the selectivity of methoctramine (METH) on neuronal muscarinic receptors, binding studies as well as muscarinic receptor-mediated responses were studied in cell aggregates from rat brain. Saturation isotherms of [3H]NMS in the presence of METH in cerebral cortex cell aggregates showed a dose dependent reduction of the *Bmax*, followed by a reduction of the ligand affinity. Furthermore, METH slowed the rate of dissociation of [3H]NMS in the same preparation, suggestive of negative cooperativity. In cortical cell aggregates prelabeled with [3H]*m*-inostol METH at micromolar concentrations induced a *stimulation* of [3H]inositol-phosphates formation in the absence of carbamylcholine (CBC), that was not inhibited by atropine. Within the concentration range of METH that did not stimulate PI metabolism, Schild plots showed a pA_2 of 6.4. METH also showed non-competitive effects at micromolar concentrations on the cAMP inhibition by CBC in caudate cell aggregates (pA_2= 7.3). Our data indicate that METH acts as a competitive antagonist only at low doses, and that it has poor selectivity for neuronal muscarinic receptor subtypes (m1 and m4) that mediate IP metabolism and cAMP inhibition.

515.19

NOVEL SUBTYPE-SELECTIVE MUSCARINIC AGONISTS. K. A. Jacobson, B. Bradbury, and J. Baumgold. Lab. of Chemistry, NIDDK and MBS, Lab of Mol. and Cell. Neurobiology, NINDS, NIH, Bethesda, MD 20892.

A series of analogues of the muscarinic agonist BM5 were synthesized using the functionalized congener approach to drug design (Jacobson et al., Mol. Pharmacol. 29 126, 1986). These compounds were evaluated for agonist activity at the second-messenger level in a series of cell lines that each express a single muscarinic receptor subtype. Compound BM5 was found to stimulate only the adenylate cyclase-coupled muscarinic receptors, subtypes m2 and m4. It behaved as an antagonist at the PI turnover coupled receptor subtypes, m1 and m3. Therefore, BM5 is a subtype-selective muscarinic agonist. Since BM5 has been described as a post-synaptic agonist and a pre-synaptic antagonist (Nordstrom et al., Mol. Pharmacol. 24 1-5, 1983), these studies indicate that post-synaptic muscarinic receptors are adenylate cyclase coupled, whereas pre-synpatic muscarinic receptors are coupled to PI turnover. Like BM5, several of the novel compounds were also subtype-selective partial agonists in that they stimulated only the m2 and m4 subtypes and were inactive as agonists at m1 or m3 receptor subtypes. These compounds are being evaluated further for their potential in treating the cognitive deficits of Alzheimers disease.

515.20

EFFECT OF GTP AND GppNHp ON AGONIST-INDUCED COUPLING OF MUSCARINIC RECEPTORS (mAChR) TO G PROTEINS. D.F. Matesic* and G.R. Luthin (Spon: G.C. Salmoiraghi). Dept. of Physiology & Biophysics and Institute for Neuroscience, Hahnemann University, Philadelphia, PA 19102.

Complexes of mAChR and G protein induced by carbachol were solubilized from rat cardiac membranes and purified using sequential lectin affinity chromatography and precipitation employing cardiac-selective anti-mAChR antibodies. G protein subunits that co-purified with agonist-labelled mAChRs were separated by SDS-PAGE and visualized on Western blots with anti-G protein antibodies. Either GTP or GppNHp reversed the mAChR-G protein interaction when included during incubation of agonist with membranes and throughout solubilization/ purification. When added to membranes only GppNHp, but not GTP, reversed the mAChR- G protein interaction. In contrast, following membrane solubilization GTP, but not GppNHp, effected uncoupling of mAChR- G protein complexes. Comparison of the kinetics of reversal of ^{3}H-agonist binding with the reversal of mAChR-G protein coupling by GTP or GppNHp indicate that mAChR-G protein complexes can exist in the absence of bound agonist. This suggests that agonist-mAChR and mAChR-G protein uncoupling by guanine nucleotides may occur as independent processes. (Supported by NS23006).

515.21

MUSCARINIC RECEPTOR-G PROTEIN INTERACTIONS IN HEART. G.R. Luthin and D.F. Matesic*. Dept. of Physiology and Biophysics and Institute for Neuroscience, Hahnemann University, Philadelphia, Pa. 19102.

Membranes from rat heart were incubated with muscarinic cholinergic receptor (mAChR) agonists or antagonists, then solubilized using digitonin/cholate. mAChRs purified by WGA affinity chromatography were precipitated using a cardiac- selective anti-mAChR antibody (Luetje et al., Biochem 26:6892, 1987). When mAChRs were thus isolated from carbachol (10 nM)-labelled membranes, the 39 kDa alpha subunit of Go was found to copurify with the mAChRs. The Go alpha subunit did not copurify with atropine-labelled mAChRs. In the presence of higher (1 mM) concentrations of carbachol, both 39 kDa Go alpha and 40 kDa Gi alpha subunits copurified with mAChRs. Saturating concentrations of agonists with full (carbachol), partial (pilocarpine) or no (McNA343) efficacy in the heart adenylyl cyclase assay all promoted copurification of G alpha subunits with mAChRs, though to varying levels. These results demonstrate a novel method to investigate mAChR coupling mechanisms. (Supported by NS23006).

515.22

POTENCIES OF PUTATIVE SELECTIVE MUSCARINIC RECEPTOR (MR) ANTAGONISTS IN BLOCKADE OF CARBACHOL-MEDIATED PHOSPHOINOSITIDE (PI) TURNOVER IN RAT CORTEX. L. Vella-Rountree and M. McKinney. Neuroscience Research Division, Pharmaceutical Discovery, Dept. 47W, Abbott Laboratories, Abbott Park, IL 60064

M1 receptor (M1R)-mediated PI turnover was measured in [3H] inositol-prelabeled mechanically-dissociated adult rat cortex. Pirenzepine (PZ) blocks this response to carbachol with pA2=8.2. We evaluated other selective MR antagonists for their potencies at the cortical M1R. Hexahydrosiladifenidol (HSD; pA2=7.7) and 4-DAMP (pA2=9.8) were found to be relatively potent at blocking M1R-PI. AF-DX 116 blocked the M1R-PI response with low potency (pA2=6.6). [Methoctramine (MET) is in progress]. Secoverine (SEC) was a potent inhibitor of M1R-PI (pA2=8.2). In all cases, the Schild slopes were not significantly different from unity, indicating simple competitive inhibition in the range of concentrations used. These antagonists blocked 6 nM [3H]PZ binding in cortex with potencies as follows: HSD (pKi=7.8), 4-DAMP (pKi=9.4), AF-DX 116 (pKi=6.7), MET (pKi=7.5), and SEC (pKi=9.2). Binding and response data were thus comparable except for SEC. These data (exclusive of PZ) are very similar to those we obtained recently for blockade of the M2R-mediated cyclic AMP inhibition in striatum, which we have concluded is a non-cardiac M2R.

515.23

ABSENCE OF AGE-RELATED ALTERATION IN BRAIN MUSCARINIC RECEPTOR-MEDIATED PHOSPHOINOSITIDE HYDROLYSIS AND IN ITS ATTENUATION BY PHORBOL ESTERS, TETRODOTOXIN AND RECEPTOR DESENSITIZATION. W. Surichamorn, E.A.M. Abdallah* and E.E. El-Fakahany. Dept. of Pharmacol. & Toxicol., Univ. of Maryland, Baltimore, MD 21201.

Phosphoinositide (PI) hydrolysis products have been postulated to play important roles in regulating neuronal function and in the process of memory. Therefore, the effects of aging on muscarinic receptor-mediated PI hydrolysis in various brain regions were investigated in male Fisher-344 rats. The muscarinic agonist oxotremorine-M induced a dose-dependent increase in this response to the same magnitude in the cerebral cortex, striatum, hippocampus, thalamus, hypothalamus and cerebellum in young and old animals. The EC_{50} measured in the first three brain regions were independent of age. The ability of elevated of K^+ to potentiate the response in cortex was equal in both age groups. In addition, a phorbol ester and tetrodotoxin suppressed the agonist-induced PI hydrolysis in cerebral cortex, striatum and hippocampus to the same extent in young and old rats. Moreover, there was no influence of aging on down-regulation of cell surface receptors and desensitization of receptor function in cerebral cortex after preincubation with oxotremorine-M. Therefore, PI hydrolysis in response to activation of brain muscarinic receptor does not appear to be sensitive to aging-related alterations.

515.24

THE M_1 MUSCARINIC RECEPTOR REGULATES CYTOSOLIC CALCIUM VIA A PERTUSSIS TOXIN SENSITIVE PATHWAY IN A EUKARYOTIC GENE EXPRESSION SYSTEM. J. Lai, T.L. Smith*, L. Mei, M. Ikeda, J. Gomez, W.R. Roeske and H.I. Yamamura. Dept. Pharmacol., Uni. Arizona, Tucson, AZ 85724; *Res. Svc., VAMC, Tucson, AZ 85723.

Stable expression of the muscarinic receptor encoded by the rat m_1 gene in transfected B82 cells was detected by the specific binding of [^{3}H](-)MQNB. The receptor had high affinity for pirenzepine (PZ) and low affinity for AF-DX 116. The apparent molecular weight of this receptor was 80,000 ±3,000 daltons, indicative of normal post-translational processing. The pI of the receptor was 5.0. Stimulation of this receptor in intact cells with carbachol (CCh) produced a transient, dose-dependent rise in $[Ca^{2+}]_i$, determined fluorimetrically with the calcium indicator, fluo-3. Maximum $[Ca^{2+}]_i$ elevation was 3 times the basal value (77±22 nM). The EC_{50} value of CCh was 2.8 (2.0-3.1) μM and its E_{max} value was 176 nM $[Ca^{2+}]_i$. The pseudo-Hill coefficient of this saturation isotherm was 1.1. The potency of PZ and AF-DX 116 in inhibiting this response paralleled the affinities of these ligands for the receptor. CCh had no effect on $[Ca^{2+}]_i$ in non-transfected B82 cells. A transient removal of external calcium suppressed this rise in $[Ca^{2+}]_i$ by 20-60%. These cells did not contain voltage dependent calcium channels. This CCh induced rise in $[Ca^{2+}]_i$ was inhibited by pertussis toxin at 10ng/ml ($p<0.05$). Data suggest this rise in $[Ca^{2+}]_i$ is closely linked to CCh-stimulated inositol lipid hydrolysis in these cells. Supported in part by U.S.P.H.S. grants.

515.25

DIFFERENTIAL EFFECTS OF CALCIUM ANTAGONISTS ON RAT BRAIN MUSCARINIC RECEPTOR S.Katayama*, S.Kito and R.Miyoshi (SPON: T.Kohriyama) 3 rd Dept. Int. Medicine, Hiroshima Univ. Sch. Med., 1-2-3 Kasumi, Minami-ku, Hiroshima, 734 Japan

To study functional and topographical relationship between muscarinic receptor and calcium channel, we investigated interactions of various calcium antagonists such as nicardipine, verapamil and diltiazem with muscarinic receptors using a receptor binding assay technique. Experiments were done using ^{3}H-QNB as a muscarinic ligand. Tissue homogenates were obtained from male Wistar-strain rats weighing 180-220 g.

Nicardipine, verapamil and diltiazem inhibited ^{3}H-QNB binding completely. Displacement curves of ^{3}H-QNB binding by nicardipine, verapamil and diltiazem shifted to right as concentration of ^{3}H-QNB increased in presence of different concentrations of either of these three kinds of calcium antagonists. Only the inhibition curve of nicardipine became shallower at high concentrations of the ligand. The Schild plot of nicardipine yielded curvelinear functions. This deviation from linearity of the Schild plot indicated possible allosteric interaction between muscarinic receptor and nicardipine binding site. Those of verapamil and diltiazem showed linearity with slope of 1.0 and 1.5, respectively.

515.26

FLOW CYTOMETRIC ANALYSIS OF CHOLINERGIC RESPONSES IN TRANSFECTED A9 L FIBROBLASTS. A.E. Schaffner and J.L. Barker. LNP, NINDS, NIH, Bethesda, MD 20892.

We have used voltage-sensitive indicator dyes in conjunction with flow cytometry to characterize the responses of A9 L fibroblasts, stably transfected with m1 muscarinic receptor cDNA, to cholinergic agents. Cells were removed from flasks with trypsin and allowed to recover for 2 hrs at room temperature in physiological saline. Cells were stained sequentially with the anionic dye DiBAC4(3) and the cholinergic ligand; the fluorescence (FL) intensity of 10-20,000 events was analyzed on a Becton-Dickinson FACS 440. The muscarinic agonists acetylcholine (ACh) and carbachol, at concentrations ranging from 1nM to 10 μM caused a decrease in oxonol FL indicating relative hyperpolarization of the cells. These responses were blocked by the muscarinic receptor antagonist atropine (1μM) but not the nicotinic receptor antagonist tubocurarine (50μM). Tubocurarine and atropine alone had no effect. Ion substitution experiments replacing sodium with N-methyl-D-glucamine and applying the cationophore gramicidin indicated a resting membrane potential of about -50 mV and a 20-25 mV change to a more negative potential when cells were exposed to ACh. Preliminary experiments using the calcium (Ca++) indicator dye fluo-3 along with the oxonol dye DiBAC4(5) suggested that the Ca++ signal increased during hyperpolarization. These results are in agreement with those obtained using patch-type electrophysiological recording techniques (Jones et al. PNAS, 85:4056, 1988). Thus, flow cytometric analysis of newly transfected cells can be used to determine the success of transfection for neurotransmitter receptors or other membrane proteins functionally coupled to membrane potential. The sorting capability of the instrument should make it possible to enrich for transfected cells without the necessary time and effort involved to establish neomycin resistance.

515.27

DIFFERENTIAL RESPONSIVENESS TO BM-5 (N-METHYL-N-[1-METHYL-4-PYRROLIDINO-2-BUTYNYL]-ACETAMIDE AND CARBAMYLCHOLINE IN MUSCARINIC RECEPTOR INDUCED HYPOTHERMIA AND BINDING TO RAT CNS MUSCARINIC RECEPTOR SUBTYPES. M. Watson and X. Ming*. Dept. of Pharmacology, Univ. of Med. and Dent. of New Jersey- N.J. Medical School, Newark, N.J. 07103-2757.

Pharmacologic activity of agonists is dependent on binding properties and efficacy. An oxotremorine analog, BM-5, has muscarinic acetylcholine receptor (mAChR) agonist-like effects. Yet studies in vivo show it's tremorolytic. Characterization of binding of this unique partial agonist and potent full agonist carbamylcholine (CAR) was done in rat cerebral cortex, yielding affinity (Ki) values for inhibition of [^{3}H](-)quinuclidinylbenzilate ([^{3}H](-)QNB), [^{3}H]pirenzepine ([^{3}H]PZ) and [^{3}H]AF-DX 116, as previously described. BM-5 (-4°F) induced <50% of maximal decrease in body temperature caused by CAR (-8°F). After 14d chronic (ip) BM-5 (10mg/kg/d) or CAR (1mg/kg/d), hypothermia induced by CAR was significantly less, and by BM-5 was zero. CAR causes significant ($p<.05$) mAChR downregulation or subsensitivity (64% of control [^{3}H](-)QNB binding). BM-5 causes upregulation. Further, BM-5 caused an increase (151%, $p<.05$) in [^{3}H]AF-DX 116 binding, a supersensitivity similar to that caused by chronic atropine (10mg/kg/d) or BM-5 (5mg/kg/d) measured via [^{3}H](-)QNB. BM-5 may act as an agonist or antagonist. It may have some mAChR subtype selectivity, but also has selective efficacy related to its intrinsic properties as a partial agonist at mAChR subtypes. Supported in part by FUMDNJ, BRSG & MH-43024.

515.28

INTERACTION OF ANTICHOLINERGICS WITH ACETYLCHOLINE RECEPTORS AND CORRELATION WITH ANTIHYPORTHERMIC AND BEHAVIORAL EFFECTS. L. Raveh, S. Chapman, S. Shapira, G. Cohen, E. Grauer, G. Porath, Y. Kapon and G. Amitai. Dept. of Pharmacology, IIBR, P.O.B. 19, Ness-Ziona 70450, Israel.

The antimuscarinic and antinicotinic activity of several anticholinergic compounds was studied in rat brain and in muscle cells in culture, respectively. Affinity to the muscarinic receptors (mAChR) was evaluated by competition with [^{3}H]QNB binding in whole brain homogenate. The antinicotinic action was determined by the inhibition of carbamylcholine-induced sodium influx in intact BC3H-1 muscle cells. The antimuscarinic K_i values obtained for these ligands ranged between 10^{-7} - 10^{-9}M with the following descending order of potency: scopolamine > atropine > artane (trihexyphenidyl) > apraphen > benactyzine > adiphenine. Noncompetitive inhibition of the nicotinic acetylcholine receptor (nAChR) was obtained with apraphen, benactyzine and adiphenine at the micromolar concentration level, whereas atropine blocked the response only at 150-200 μM. Antihypothermic potency was evaluated by determining the minimal effective dose (MED) which diminishes oxotremorine-induced hypothermia in rats. The most efficacious compound was scopolamine which displayed its activity at 0.1 mg/kg (s.c.), whereas the least active compound was adiphenine (>50mg/kg). Affinity for central mAChR correlated with the corresponding antihypothermic MED except for benactyzine which was more effective than expected from its affinity to mAChR. Cognitive tests using the DRL paradigm in rats displayed only partial correlation with binding to mAChR. Atropine displayed its behavioral effect at significantly higher dose than what would be expected from in-vitro experiments, whereas benactyzine was effective at a dose which was lower than anticipated from the binding studies.

515.29

CULTURED RAT HIPPOCAMPAL NEURONS EXPRESS FUNCTIONAL MUSCARINIC RECEPTORS. M.L.Fiszman*, S.V.P. Jones and J.L.Barker. (SPON: O.Choi). Laboratory of Neurophysiology, NINDS, NIH, Bethesda, MD 20892.

Muscarinic receptor expression and function was studied in cultured rat hippocampal neurons. Cultures were obtained from 18 day old embryos. Cells were plated ($2.5x10^5$cells/dish) in 35mm poly-D-lysine-coated Costar dishes, and incubated in Minimum Essential Medium, 2mM glutamine and 5% horse serum for 3 weeks in 8% CO_2 at 37°C. Binding of ^{3}H-N-methylscopolamine (^{3}H-NMS) was performed at room temperature for 30 minutes. Specific ^{3}H-NMS binding (in the presence of 1μM atropine) was demonstrated in these three week-old cultures. Electrophysiological responses to muscarinic agonists were recorded using the whole-cell patch-clamp technique. In current clamp, application of carbachol (1μM) from a pressure ejection pipette depolarized the cell membrane from about -48mV to about -28mV in 40% of cells tested. Injection of depolarizing current in cells held in current-clamp at -48mV resulted in activation of action potentials with consequent activation of the afterhyperpolarization (AHP). Carbachol (1μM) increased the action potential firing rate and completely inhibited the AHP. All three effects were blocked by 1μM pirenzepine or by 10μM atropine, showing that 3 weeks-old cultured hippocampal neurons express functional muscarinic receptors. Interestingly, carbachol often only induced one of these responses in some cells. An investigation of the putative muscarinic receptor subtype responsible for these effects is being carried out.

POSTSYNAPTIC MECHANISMS III

516.1

Effect of chronic haloperidol treatment on rat striatal calcineurin (CN) activity. E. Chung, M.T. Dvorozniak*, M.H. Van Woert and H.C. Li*. Departments of Neurology, Pharmacology and Biochemistry. Mount Sinai School of Medicine, New York, NY 10029

CN is a Ca2+/calmodulin-dependent phosphatase present in highest concentrations in the striatum. It has been suggested that CN regulates neurotransmission by turning off the cAMP signal. We have shown that CN activity is localized to intrinsic striatal neurons. Some striatal neurons contain dopamine (DA) receptors and receive nigral DA input. Since DA receptors are linked to adenylate cyclase we have investigated whether rat striatal CN activity (measured using 32P-ser-casein) is altered by DA receptor supersensitivity produced by haloperidol injections for 20 days.

	CN activity	3H-spiroperidol
control	1.71±0.13	148.6± 7.4
haloperidol		
0.5 mg/kg	1.56±0.11	188.8±10.3*
5.0 mg/kg	1.24±0.08*	181.0± 8.0*

*= p less than 0.001 compared to control.

5.0 mg/kg chronic haloperidol treatment significantly reduced striatal CN activity. (Supported by grants from Tourette Syndrome Association and NIH NS11631)

516.2

Effect of quinolinic acid lesions(QA) on rat striatal calcineurin (CN) activity. M.H. Van Woert, E. Chung, M.T. Dvorozniak* and H.C. Li* Departments of Neurology, Pharmacology and Biochemistry, Mount Sinai Sch of Med, New York, NY 10029

Intrastriatal infusion of QA, an endogenous excitotoxin, is thought to mimic the neurochemical and histopathological features of Huntingtons disease. CN is a Ca2+/calmodulin-dependent phosphatase present in highest concentration in the striatum. We have investigated the effects of varying doses of QA used to lesion the striatum on CN activity (measured using 32P-ser-casein). In order to estimate the extent of intrinsic neuronal cell body loss, GAD and CAT activites in the same striatal tissue also were measured.

	CN	GAD	CAT
control	1.63±0.12	122±10	77±4
QA 30 nmoles	1.14±0.09*	77±4*	68±3
QA 90 nmoles	0.76±0.05*	41±4*	49±3*
QA 270 nmoles	0.79±0.07*	47±9*	44±3*

* = P less than 0.001 compared to control

With 30 nmoles QA, the reduction of CAT activity was not statistically significant, while CN and GAD activities were decreased significantly. At 90 and 270 nmoles of QA, striatal CAT, GAD and CN activities were all decreased. (Supported by grant NS11631)

516.3

IN VIVO [Na^+, K^+]-ATPASE ACTIVITY IN *NARCINE BRASILIENSIS*. H. Blum* and R.G. Johnson, Jr.*(SPON: M. Reivich).H.Hughes Med. Inst., Univ. of Penna Sch. of Med., Phila, PA 19104.

We have measured the *in vivo* activity of the electric organ [Na^+, K^+]-ATPase in the dorsal membrane of the electrocyte of *Narcine brasiliensis* noninvasively using 31P and 23Na nuclear magnetic resonance spectroscopy. Activation of the sodium pump induced by electrical stimulation of the organ can be accurately quantitated by monitoring the fall of phosphocreatine (PCr), a high energy phosphagen in the electrocyte. This depletion is blocked by preincubation in the cardiac glycoside ouabain. The [Na^+, K^+]-ATPase rate increases by a factor of at least 2000 with normal stimulation so as to maintain intracellular sodium ($[Na^+]_i$) unchanged until virtually full depletion of PCr. Recovery of $[Na^+]_i$ is accomplished in under 2 sec. However, incubation of excised slices of electric organ under conditions that will increase $[Na^+]_i$, including hyperosmolar buffer, gramicidin, and monensin failed to increase PCr turnover. In contrast, tetraphenylphosphonium bromide, which collapses the transmembrane potential, does activate the [Na^+, K^+]-ATPase, even after preincubation in the nicotinic antagonist d-curarine chloride. It thus appears that activation of the sodium pumps in this tissue may depend on a signal associated with the ventral membrane rather than sodium concentration alone. The results suggest that *Narcine* may be an excellent model for study of Na^+ pump activation in an excitable tissue.

516.4

PROTEOLYTIC ACTIVATION OF CaM-KINASE II: PUTATIVE FUNCTION IN SYNAPTIC PLASTICITY. D.P. Rich*, C.M. Schworer*, R.J. Colbran*, & T.R. Soderling. Dept. of Molecular Physiology & Biophysics, Vanderbilt University, Nashville, TN 37232

Rat forebrain CaM-kinase II in isolated postsynaptic densities (PSD) was subjected to limited proteolysis by μ-calpain, a Ca^{2+}-dependent protease. Incubation of autophosphorylated or Ca^{2+}/CaM-bound kinase with this protease resulted in solubilization of a Ca^{2+}/CaM-independent kinase activity which was 3-5 fold greater than the initial Ca^{2+}/CaM-independent activity in the PSD. Western blot analysis using polyclonal antibodies to soluble kinase holoenzyme indicated that μ-calpain generated several immunoreactive fragments between 21-32kDa. μ-Calpain, however, only degraded a small fraction of the intact kinase subunits. [^{125}I]CaM-overlays indicated a major CaM-binding fragment of 23kDa in μ-calpain digests of CaM-kinase II. This peptide was shown to contain the regulatory autophosphorylation site (Thr-286) of the kinase. Immunoblotting with antibody raised to a synthetic peptide from the catalytic domain of the kinase indicated that there was a single active fragment of approximately 30kDa in the μ-calpain digests. Analysis of crude digests using Superose 6 gel filtration also indicated that Ca^{2+}/CaM-independent activity resided in a 30kDa fragment. Thus, μ-calpain appears to cleave CaM-kinase II into a 30kDa catalytic domain fragment and a 23kDa regulatory domain fragment. These data support a putative mechanism for persistent regulation of synaptic events by such proteolytic activation of CaM-kinase II.

516.5

A Ca^{2+}-DEPENDENT, LONG-LASTING HYPERPOLARIZATION INDUCED BY INOSITOL TRISPHOSPHATE (IP_3) IN CAT SPINAL MOTONEURONS. L. Zhang and K. Krnjević, Dept. Anaesthesia Res. and Physiology. McGill University, Montréal, Québec, Canada H3G 1Y6.

In experiments on pentobarbital-anaesthetized cats or decerebrate cats, intracellular iontophoresis of IP_3 (3-10 nA for 0.5-1 min) induced a reproducible, long-lasting hyperpolarization (2-16 mV), with unchanged input resistance. The hyperpolarization started 0.5-1 min after the end of injection, reached its maximum within 5 min, and then persisted for another 10-60 min. Associated with the hyperpolarization, there were increases in the spike size and rate of rise, and in EPSP amplitude, but the post-spike after-hyperpolarization (AHP) was unchanged or increased in amplitude. Repetitive discharges, evoked by intracellular depolarizing currents, were reduced during the hyperpolarization. Simultaneous injections of IP_3 and BAPTA did not induce any hyperpolarization, whereas the Ca^+-dependent AHP was depressed by 60%. The effects of IP_3 closely mimic the long-lasting hyperpolarizing action of 5-HT (probably mediated by 5-HT_2 receptors) on these motoneurons, in their slow time course and Ca^+-dependence, suggesting that IP_3 may be an intracellular mediator of 5-HT action.

Supported by MRC of Canada.

516.6

SYNAPTIC TRANSFER FUNCTION OF GIANT AXON TERMINAL-SPINAL NEURON OF THE LAMPREY. L.E. Moore and J.T. Buchanan.* Dept. Physiology & Biophysics, Univ.Texas Med. Br., Galveston, Texas 77550.

Synaptic potentials and transfer impedance functions of the Müller giant axon terminal-motoneuron synapse were measured by placing one intracellular electrode near the pre-synaptic terminal and a second electrode in the soma of the post-synaptic neuron. During block of the chemical synapse in zero calcium the axon remains electrotonically connected through gap junctions providing a direct access to the dendritic tree from the electrode in the axon. It was shown that the electrotonic synaptic potential was enhanced by depolarization of the post-synaptic membrane potential. Similar depolarizations caused an increase in the membrane impedance using a white noise single electrode transfer function analysis. This effect was abolished by 1 μM TTX suggesting that a subthreshold voltage dependent negative sodium conductance is responsible for the enhancement of synaptic potentials with depolarization. A synaptic transfer function was used to estimate the synaptic location on an equivalent dendritic cable and calculate the synaptic potentials for different membrane potentials.

Supported in part by NIH-2P01-NS-11255.

516.7

AMINO ACID RESPONSES OF LAMPREY SPINAL NEURONS. J.T. Buchanan*, B.N. Christensen, and L.E. Moore (SPON: H.A. Lekan). Department of Physiology and Biophysics, University of Texas Medical Branch, Galveston, TX 77550

Lamprey spinal neurons, either isolated or within the spinal cord, were tested with excitatory and inhibitory amino acids. The response of enzymatically isolated neurons to the excitatory amino acids quisqualate (QA) and n-methyl-d-aspartate (NMDA) were measured with whole-cell patch clamp electrodes. Both QA and NMDA induced an inward membrane current with a reversal potential near 0 mV indicating that they open cation selective channels. QA responses had an EC_{50} and Hill coefficient of 4.3 μM and 2.2, respectively, the latter indicating cooperativity in QA binding. NMDA currents were reduced by extracellular Mg^{++}, and the current-voltage curve in normal Mg^{++} displayed a negative-slope conductance near the resting potential. NMDA responses were potentiated by glycine with an EC_{50} and Hill coefficient of 1.4 μM and 1.7, respectively. Measurements of impedance functions with a white noise technique revealed that the excitatory amino acids glutamate and QA caused a slight decrease in the membrane impedance of both isolated neurons and intact motoneurons. In contrast, NMDA induced a pronounced increase in the impedance consistent with the negative-slope conductance observed in the NMDA current-voltage curve. The inhibitory transmitters glycine and GABA decreased the impedance over a wide frequency range.

Supported by NS-11255 from DHHS.

516.8

AFTERHYPERPOLARIZATION (AHP) CHANGES AND VOLTAGE-ADDRESSABLE INFORMATION PROCESSING DERIVED FROM SIMULATED EFFECTS OF gK^+_A. R.G. Pay and C.D. Woody, BRI and MRRC, UCLA Med. Ctr., Los Angeles, CA 90024.

Changes in AHPs with conditioning, epilepsy, hypothalamic stimulation, and membrane modulators have been attributed mainly to modulations of calcium-dependent potassium channels or $GABA_B$. Connor & Stevens (J. Physiol, 1971) pointed out that activation-inactivation properties of an A-current could modulate membrane potentials after depolarization. A-currents have been found in 90% of mammalian (cat) motor cortex neurons (Woody et al., Br Res, 1989); thus their contribution to AHP modulations in mammalian cortical neurons merits examination. We therefore simulated time constant distributions matching observed inactivation curves of gK^+_A. The results yielded variations of AHPs as profound as those attributed to gK_{Ca} and $GABA_B$.

Review of de-inactivation curves after sustained potential shifts suggested a family of activation curves varying with sustained membrane potential. Simulation of an activation-curve family of this type, related by a decreasing, voltage-dependent sigmoidal function, indicated that dynamic interplay between A-currents and PSPs could yield Markovian-like information processing within dendrites wherein a PSP would select the current activation curve for the next PSP. A-current changes with conditioning (Alkon, Science, 1979) will thus produce a form of voltage-addressable information processing, in which the memory domains accessed depend on the temporal patterns of dendritic potential.

516.9

POSSIBLE DUAL EFFECT OF SYNAPSES THAT ARE PUTATIVELY PURELY EXCITATORY OR PURELY INHIBITORY: BASES IN STABILITY THEORY AND IMPLICATIONS FOR NEURAL NETWORK BEHAVIOR. R. Davenport*, E. Jakobsson* and B. Gerber* (SPON: F. Delcomyn) Dept. of Physiol. and Biophys., Progs. in Neur. and Behav. Biol. and Bioeng., Univ. of Ill., Urbana, IL 61801

Depolarization of an excitable membrane has a dual effect: excitatory in that it causes rapid opening of calcium and/or sodium channels but inhibitory in that it also causes those channels to inactivate. We considered whether apparently paradoxical or dual behavior might be exhibited by excitatory and inhibitory synaptic inputs. We used the classic Hodgkin-Huxley model for voltage-gated channels plus leakage channels of appropriate selectivity for ligand-gated post-synaptic channels. We summarize a model cell's behavior by calculating elicited firing frequency as a function of reversal potential and conductance of summed synaptic inputs, using stability theory and direct simulations. Dual behavior is elicited in the model with reasonable densities of ligand-gated channels. Thus a particular synaptic input to a neuron may be either excitatory or inhibitory depending on simultaneous activity of other synaptic inputs to the cell. This input-output map may give rise to biologically realistic and rich behaviors as an element of computed neural networks, and still be computationally tractable.

516.10

WHOLE-CELL VOLTAGE-CLAMP OF SYNAPTIC CURRENTS RECORDED FROM MORPHOLOGICALLY IDENTIFIED NEURONS IN SLICES OF RAT NEOCORTEX. A.R. Kriegstein, M.G. Blanton, and J.J. LoTurco, Dept. of Neurology and the Neurosciences Program, Stanford Univ. Sch. of Med., Stanford, CA 94305.

We have characterized evoked and spontaneous synaptic currents in neocortical neurons with whole-cell voltage-clamp recordings. Visual and somatosensory cortices from 20-40 day old rats were cut with a vibratome (400 uM), affixed to the bottom of petri dishes with plasma-thrombin clots, and superfused with artificial cerebrospinal fluid. Gigaohm seals were formed by plunging recording pipettes (5 Megaohms) into the slices, clearing the tips with a pulse of positive pressure, and advancing the electrodes slowly to form partial seals (100-200 Megaohm). Suction was then applied through the electrodes to form gigaseals (2-7 Gigaohm) and additional suction was applied to rupture the underlying membranes and obtain whole-cell recordings. The input impedances for neurons recorded with this technique were 0.5 to 1.5 gigaohms. Neurons were labeled intracellularly with either Lucifer Yellow CH or biocytin.

We recorded from both pyramidal and nonpyramidal neurons in layers II-VI. Spontaneous synaptic events occurred at high frequencies, were not reduced by 0.5 uM TTX, and could be resolved as single or multiple events. Most of the spontaneous currents reversed in direction at the chloride equillibrium potential and were blocked by bath application of 5 uM bicuculline methiodide (BMI). The spontaneous events that were still present in BMI reversed at or near the equilibrium potential for monovalent cations (4 mV). Stimulation of the white matter, in 5 uM BMI, evoked a large conductance current that often had two components, and both reversed near 4 mV. Addition of 20 uM d-APV greatly reduced the duration and amplitude of the first component and blocked the second.

516.11

WHOLE-CELL PATCH-CLAMP RECORDINGS IN ADULT RAT HIPPOCAMPAL SLICES. I. Mody and T. S. Otis, Dept. of Neurology and Neurological Sciences, Stanford University Medical Center, Stanford, CA 94305.

The advantages of the patch-clamp technique have been exploited in thin (<130 μm) mammalian brain and spinal cord slices where neurons could be identified with the aid of Nomarski water immersion optics (Konnerth et al., *Soc. Neurosci. Abs.*, 14: 1046, 1988). We have employed a simplified version of the above technique to achieve patch-clamp recordings in standard (400 μm thick) hippocampal slices maintained at 35±0.5°C in a conventional recording chamber.

Patch electrodes (2-2.5 μm diameter; 4-5 MΩ), usually filled with (in mM) K-methyl-sulphate 120, $MgCl_2$ 2, HEPES 10 (pH=7.2), were lowered into any of the cell body layers of the slice under visual guidance through a dissecting microscope. Seals formed (>1 GΩ) within the upper 50 μm of the slice. Following break-through, an Axoclamp 2A was used for current- or voltage-clamp recordings. In over 30 CA1 pyramidal (PCs) and dentate gyrus granule cells (GCs) the resting membrane potentials (RMPs) ranged between 64-72 mV (PCs) and 68-80 mV (GCs); input resistances (R_Ns) were 70-130 MΩ (PCs) and 100-220 MΩ (GCs). All neurons had overshooting action potentials with a threshold of 30-40 mV more positive to rest. The RMPs are in good agreement with previous studies using sharp electrode recordings. However, the R_Ns are consistently 4-5x larger than those reported with conventional intracellular microelectrodes. This discrepancy can be attributed to the less invasive nature of the patch-clamp recordings which produce minimal damage to cell membranes, thus avoiding leak conductances.

In summary, the patch-clamp recordings can easily be adapted to conventional preparation and recording techniques in hippocampal slices. Based on its advantages, the patch technique is anticipated to replace sharp electrode recordings in brain slice preparations.

Supported by NIH grants NS12151 and RR05353-27 to I.M.

516.12

MEASUREMENT OF INTRACELLULAR CALCIUM BY FURA INJECTION IN PYRAMIDAL CELLS OF HIPPOCAMPAL SLICES. M. Delay, D. Hochman and B.A. MacVicar (Sponsor D. Burnard), Department of Medical Physiology, University of Calgary, Calgary, Alberta.

Intracellular calcium is an important signal controlling neuronal excitability and probably has a role in inducing neuronal death during some neurodegenerative diseases. We have developed a method by which we can measure intracellular calcium in hippocampal pyramidal cells in in vitro slices while simultaneously recording activity. This has allowed us to determine changes in intracellular calcium during spike activity and also during seizure activity in slices. Slices (300μm) were isolated from the hippocampus of rats (day 21-28) and were transferred to a recording chamber on an inverted microscope for staining and recording. Fura-2 (10mM in 0.2M Kacetate, 10mM HEPES, pH 7.2) was intracellularly injected into pyramidal neurons. Fluorescence images of cells were obtained using a SIT camera and digitized with IT 151 imaging analysis system. Fluorescence intensity ratios of cells at 360/380 or 340/380 wavelengths (nm) were calibrated by comparison with either buffered Ca^{++} solutions or after application of A-23187 to obtain RMax. Bursting activity induced by either current injection or application of convulsants was correlated with increased dendritic Ca^{++}.

516.13

HYPOMAGNESIA IN NEOCORTEX MODIFIES THE RESPONSES TO ACETYLCHOLINE, GLUTAMATE & GABA. H. El-Beheiry & E. Puil, Depts. of Anaesthesia and Pharmacology & Therapeutics. University of British Columbia, Vancouver, B.C., Canada.

Clinically, hypomagnesemia produces, by an unknown mechanism, manifestations of central nervous system irritability. Experimentally, hypomagnesemia was simulated by sequential changes in external [Mg] in *in vitro* slice preparations of sensorimotor cortex. Mg-free perfusion induced a small hyperpolarization (3-5 mV) with a 10% decrease in input resistance, slow depolarizing waves insensitive to tetrodotoxin (TTX) and a TTX-sensitive enhancement of the background synaptic activity. Gradual reduction in the external [Mg] was associated with corresponding reductions in the depolarizations evoked by dendritic applications of acetylcholine or GABA. However, glutamate responses were not affected whereas responses evoked by NMDA were potentiated. Increasing external [Mg] in slices that had been incubated in Mg-free oxygenated solution enhanced the actions of acetylcholine and GABA and did not produce significant alterations of the glutamate responses. The results suggest that the effects of hypomagnesemia on transmitter actions may involve pre- and post-synaptic mechanisms.

516.14

FIRING PROPERTIES OF HUMAN NEOCORTICAL NEURONS. D. Frederick*, C. J. Wilson, A.R. Wyler*, and R.C. Foehring (Spon: D.M. Desiderio). Depts. of Anatomy and Neurobiology, and Neurosurgery, Univ. of Tenn., Memphis, TN 38163.

Human neocortical neurons were studied in a slice preparation from the midddle temporal gyrus. Intracellular recording revealed two classes of response to sustained current injection. Regular-spiking neurons (75% of 42 cells) fired repetitively throughout a 1s current injection, regardless of prior holding potential. Bursting neurons (25% of sample) responded to similar stimuli with a burst of 2-3 spikes riding upon a slow depolarizing potential, then were quiescent. Bursting was a voltage-dependent behavior: neurons showed a burst response from holding potentials of approximately -65 mV (or negative) but fired repetitively when held at depolarized potentials. Bursting was also dependent upon stimulus intensity: at hyperpolarized potentials a large stimulus resulted in repetitive firing. Bursting neurons exhibited a larger afterdepolarization following an action potential than regular-spiking neurons (only significant difference). Several cells were filled with biocytin and later stained to determine neuronal morphology. Both firing types were observed in pyramidal cells. Variability of firing pattern suggests that busting and regular-spiking may be extremes of a continuum of firing behavior.

516.15

EFFECTS OF DEPOLARIZING PULSE POTENTIALS ON THE FIRING RATE OF CAT NEOCORTICAL NEURONS A.D. Reyes*and E.E. Fetz (SPON: M. Mustari) Dept. of Physiology & Biophysics and Primate Res. Ctr., Univ. of Wash., Seattle, WA 98195

Excitatory post-synaptic potentials (EPSP's) produce an increase the firing rate of active neurons by advancing the occurence of threshold crossings in the interspike interval (ISI). We investigated the underlying mechanisms by probing the ISI with depolarizing pulse potentials (PP's), which have been shown to mimic the effects of EPSP's on neuronal firing.

Intracellular recordings were obtained from layer V pyramidal cells in slices of cat sensorimotor cortex. To mimic EPSP's, brief current pulses were injected through the recording electrode, producing PP's with a near-linear rise of membrane potential followed by an exponential decay (in resting neurons). During repetitive discharge, induced by injection of d.c. current, the PP's were injected at random intervals. Offline computer analyses calculated the average ISI shortening produced by PP's at different delays and their contributions to the cross-correlograms.

PP's were found to shorten the ISI in two ways. During the last part of the ISI, PP's crossed threshold (which is an increasing function of time) and produced counts in the correlogram peak. At earlier times in the ISI, PP's produced delayed threshold crossings that also shortened the ISI, apparently due to a persistent sodium current; these PP's contributed to cross-correlogram counts beyond the peak. Preliminary observations suggest that, in many cells, the second effect can contribute more to the overall change in firing rate than the direct threshold crossings.

516.16

INWARD RECTIFICATION VARIES WITH CORTICAL LAMINA IN GUINEA PIG NEOCORTICAL NEURONS IN VITRO. A. Williamson and D. A. McCormick, Sect. Neuroanatomy, Yale Univ. Sch. Med., New Haven, CT 06510.

Two forms of inward rectification have been described in neurons: the first is a K^+-dependent current which activates hyperpolarized to E_K and which is sensitive to both extracellular Cs^+ and Ba^{++}. The second, known as I_h or I_Q, is a mixed Na^+-K^+ current that activates at membrane potentials negative to -65 mV and can be readily blocked by Cs^+ but not by Ba^{++}. Here we studied the rectification properties of pyramidal cells in different lamina of cingulate, somatosensory, and temporal cortical slices maintained *in vitro*.

We found that these two rectifiers are present in a lamina specific manner. Local application of Ba^{++} (100-500 uM) produced similar effects in both layer II-III and layer V cells, namely a non-rectifying inward current and the block of a rectifying, presumed K^+, inward current whose average point of activation was -98.5 mV. In contrast, application of Cs^+ (2-3 mM) to layer II-III cells resulted in block of only the current activated at membrane potentials negative to E_K (average point of activation: -96.5 mV), while in layer V cells, Cs^+ also blocked I_Q, which activated at membrane potentials negative to -64 mV. In current clamp recordings these differences were expressed as the presence (most layer V, and all burst-generating, neurons) or absence (layer II-III cells) of a depolarizing "sag" upon hyperpolarization of the membrane potential. These data indicate that there are at least two distinct anomalous rectifiers which are non-homogenously distributed among neocortical pyramidal cells. This finding is in agreement with the hypothesis that subpopulations of cortical pyramidal cells possess distinct morphological, electrophysiological and pharmacological properties. Supported by NINCDS, Jacob Javits Center for Neuroscience, and the Klingenstein Foundation.

516.17

INWARD RECTIFICATION OF PROJECTION CELLS IN THE RAT AND CAT LATERAL GENICULATE NUCLEUS. S. Lightowler*, J.W. Hynd*, C.E. Pollard* and V. Crunelli. Dept. Pharmacology, St.George's Hospital Medical School, London SW17 ORE, UK.

Thalamic projection cells in vivo and in vitro often dispaly rhythmic membrane potential oscillations which are partly due to the activation of time- and voltage-dependent conductances. We have now studied the properties of a hyperpolarization activated current using the single electrode voltage-clamp technique in brain slices of the dorsal lateral geniculate nucleus (150mM Na^+, 0.5mM Ca^{2+}, 3mM Mg^{2+}, 6.25mM K^+, 1mM Ni^{2+} and 0.5μM TTX).

Hyperpolarizing voltage steps from holding potentials of -45 to -55mV evoked a slow non-inactivating inward current that was followed by an inward tail current on repolarization to the holding potential. The current was activated over the range -55 to -110mV and tail current analysis showed a reversal potential of -31.1 ± 1.7mV. This value was shifted to more negative potentials by reducing either $[Na^+]o$ or $[K^+]_o$, and to more positive potentials by increasing $[K^+]_o$. The current was reversibly abolished by 3mM Cs^+ but relatively resistant to 4mM Ba^{2+}.

We conclude that thalamic projection cells possess a hyperpolarization-activated inward current carried by Na^+ and K^+ ions and thus similar to the neuronal I_Q, I_H and the cardiac I_F. The properties of this current indicate that it might contribute to the oscillatory activity of thalamic projection cells.

516.18

PHYSIOLOGICAL CHARACTERISTICS OF A VERY SLOW EXCITATORY POST-SYNAPTIC POTENTIAL IN THE LGNd. H.R. Feeser, H.-C. Pape, and D.A. McCormick Section of Neuroanatomy, Yale University School of Medicine New Haven, CT

Transmission of visual information from the retina to cerebral cortex is modulated by slowly acting neurotransmitters in the lateral geniculate nucleus (LGNd). Although the ionic mechanisms by which these substances alter the excitability of LGNd neurons has been investigated, the possibility that these actions may occur after physiological release of the substance has not. Therefore, we investigated this possibility with intracellular, *in vitro* techniques applied to guinea pig LGNd slices. Local electrical stimulation resulted in a slow excitatory PSP whiched ranged from 2-15 mV in amplitude and 5 sec to >1 min in duration. This slow EPSP is present after just one stimulus, but increases in both duration and amplitude following trains of stimuli. Local application of TTX blocks this response suggesting that the slow EPSP is mediated by the synaptic release of a neuroactive substance. The slow EPSP is associated with an increase in apparent input resistance, becomes larger in amplitude at depolarized membrane potentials and exhibits non-additivity with the NE- and ACh-induced slow depolarizations. These results suggest that the slow EPSP, like the responses to ACh and NE, is mediated by a decrease in a resting potassium conductance. A substantial slow EPSP could still be evoked even after block ofα1,α2,β, and 5-HT receptors. The reduction or block of this slow EPSP by scopolamine indicates the presence of a substantial cholinergic component. These results reveal that release of neuroactive substances by nerve terminals in the LGNd can cause prolonged changes in the excitability of thalamocortical relay neurons. Supported by NINCDS.

516.19

NMDA AND NON-NMDA RECEPTORS MEDIATE BOTH HIGH AND LOW FREQUENCY SYNAPTIC POTENTIALS IN THE RAT LATERAL GENICULATE NUCLEUS. I. Soltesz*, M. Haby*, D. Jassik-Gerschenfeld*, N. Leresche*, and V. Crunelli (Spon: ENA). Dept. Pharmac., St.George's Med. Sch., London, UK; MRC Anat. Neuropharm. Unit, Dept. Pharmac., Oxford, UK; Inst. Neurosc. Vision, Univ. Curie, 4 Jussieu, Paris, France.

We have re-investigated the relative contribution of NMDA and non-NMDA receptors to the EPSPs evoked in projection cells of the rat lateral geniculate nucleus by low and high frequency stimulation of the optic tract.

In the presence of Mg^{2+}, 6-cyano-7-nitroquinolaxaline-2,3-dione (CNQX), at concentrations selective for the non-NMDA receptors (5-10μM), reversibly reduced (85±8%) the EPSP evoked at low frequency stimulation (0.03-0.05Hz). In the continuous presence of CNQX, the remaining EPSP was enhanced by removing Mg^{2+} from the perfusion medium, and abolished by the NMDA antagonist DL-2-amino-5-phosphono-pentanoic acid (APV) (20μM). In the same cells, however, APV applied before CNQX had no effect on the EPSP.

High frequency stimulation (50-100Hz, 200-500 msec) performed in the presence of 1-2mM Mg^{2+} evoked fast EPSPs (at each shock) superimposed on a slow depolarization. APV reversibly reduced (45-80%) the slow depolarization with no effect on the fast EPSPs. These results indicate the involvement of both NMDA and non-NMDA receptors in the retinal input to the LGN and are consistent with results recently obtained in cat X and Y cells in vivo (Murphy, Salt & Sillito, Physiol. Soc. Meet., London, April 1989).

516.20

SIMULATION OF EVOKED FIELD RESPONSES. L. STAN LEUNG. DEPT. CLIN. NEUROL. SCI. AND PHYSIOLOGY, UNIV. WESTERN ONTARIO, LONDON, CANADA N6A5A5.

Field potentials in a cortical structure (e.g. hippocampus) were simulated by a three-step procedure: (1) Intracellular responses using the Rall's compartment model, (2) current-source-densities and then (3) field responses. This approach allows direct comparison with experimental data. A cylindrical population of uniformly activated neurons and a homogeneous isotropic medium were assumed. Dipole fields were generated by discrete somatic or dendritic synaptic inputs represented by t*exp(-at) functions. Proximal dendritic excitation gave mirror-image like field potentials across a zero isopotential layer, while distal excitation was associated with a 'reversal zone' (about 300um depth) of biphasic responses. Positive fields generated by excitatory inputs were larger for distal than proximal inputs. A 8-mV somatic IPSP gave a field about 2 times smaller than a 8-mV somatic EPSP, probably because the time course of the inhibitory input was assumed to be 10 times slower than the excitatory one. However, large open (dipole) fields were generated by somatic or dendritic IPSPs. Supported by NSERC and MRC.

CATECHOLAMINES

517.1

THE EFFECT OF α-METHYL-*p*-TYROSINE ON THE CONCENTRATION OF β-PHENYLETHYLAMINE IN THE RAT STRIATUM. I.A. Paterson, A.V. Juorio* and M.-Y. Zhu*. Neuropsychiatric Res. Unit, Univ. of Saskatchewan, Saskatoon, Sask.,Canada. S7N 0W0.

β-Phenylethylamine (PE) is an endogenous brain amine which is present in the striatum at a concentration of 3.3 ng/g. PE is synthesized by 6-hydroxydopamine sensitive neurones which arise or pass through the substantia nigra (SN) and it has been shown that stimulation of the SN reduces the concentration of PE in the ipsilateral striatum in deprenyl treated rats. The object of these experiments was to determine the effect of inhibition of tyrosine hydroxylase (TH) on the concentration of PE following SN stimulation. The effect of stimulation of the SN (20 Hz, 100 μA, 1 hour with concentric bipolar electrodes) on the striatal accumulation of PE was determined in deprenyl-pretreated (2mg/kg, i.p., 3 hours), urethane (1 g/kg, i.p.) anaesthesized rats. The determination of PE was performed by a mass spectrometric technique and DA, DOPAC and HVA by HPLC with electrochemical detection. The stimulation of the SN produced an increase in the turnover of DA and a significant reduction in the concentration of striatal PE. The administration of α-methyl-*p*-tyrosine (1.25 mg/kg, i.p., 2 hours) did not affect the concentration of PE in unstimulated striata but prevented the stimulation-induced decrease in the concentration of PE. These experiments demonstrate that the rate of accumulation of PE is decreased by the activation of TH and therefore, PE is present in TH-containing neurons. We conclude that PE coexists with DA in dopaminergic neurones in the nigrostriatal pathway. Supported by Saskatchewan Health and the MRC of Canada.

517.2

THE EFFECTS OF A BENZODIAZEPINE PARTIAL AGONIST, RO 16-6028, ON BASAL AND STRESS-INDUCED IN VIVO TYROSINE HYDROXYLATION IN THE MESOTELENCEPHALIC DOPAMINE SYSTEM. K. Hirada* ,A. Y. Deutch[1], and M. Goldstein (SPON: J. E. Platt). Department of Psychiatry, New York University School of Medicine, New York, NY 10016 and [1]Dept. of Psychiatry, Yale Univ. Sch. Med., New Haven, CT 06508.

Anxiolytic benzodiazepine (BZD) agonists decrease dopamine (DA) turnover in mesotelencephalic DA terminal fields, and prevent a stress-induced augmentation of DA turnover in the prefrontal cortex (PFC). Ro 16-6028, a novel BZD partial agonist, possesses the anticonvulsant and anxiolytic properties of typical BZD agonists, but exhibits greatly reduced muscle relaxant and sedative properties relative to full BZD agonists (J. R. Martin et al., Pharmacopsychiat 21:360, 1988). We therefore examined the effects of Ro 16-6028 on in vivo tyrosine hydroxylation (an index of DA synthesis). Ro 16-6028 effected a dose-related decrease in DA synthesis in mesotelencephalic DA terminal fields; at the highest dose tested (5.0 mg/kg, ip) DOPA accumulation returned to control levels in the PFC and striatum, but not other regions. The most potent effects of Ro 16-6028 were observed in the ventral tegmental area (VTA); moreover, Ro 16-6028 prevented a stress-induced augmentation in tyrosine hydroxylation in the VTA. These data may suggest that the muscle relaxant properties of BZD agonists are not essential for anxiolytic action.

517.3

SOLUBILIZATION AND PARTIAL PURIFICATION OF THE [^{3}H] GBR-12935 BINDING SITE/DOPAMINE REUPTAKE CARRIER PROTEIN FROM RAT STRIATUM. P.Berger, R.Martenson, K.C.Rice[1], A.Thurkalf[1], S.M.Paul (SPON:J.Contrera). Clinical Neuroscience Branch, NIMH, Bethesda, MD 20892, and [1]NIADK, Bethesda, MD 20892.

The binding of a series of tritiated ligands including GBR-12935, cocaine, nomifensine, mazindol and methylphenidate, to the dopamine transporter protein has been reported. These radioligands bind in a sodium dependent manner and there is a good correlation between inhibition of radioligand binding and dopamine uptake. Among this series of compounds [^{3}H] GBR-12935 is the most selective for the dopamine transporter (Berger et al, 1986). Consequently, we have used this ligand to monitor the solubization and purification of the dopamine reuptake carrier from rat striatal membranes.

Our initial screening of several detergents revealed optimal solubilization efficiency and stability of the solubilized binding site using the zwitterionic detergent CHAPS. After solubilization in CHAPS (0.5%) followed by ultracentriguation (200,000g) the specific binding of [^{3}H] GBR-12935 in the supernatent closely resembles that in membranes. Specific binding was sodium-dependent and good correlations were observed between the inhibition of binding in the solubilized and membrane preparation (r=.99). Moreover, the potency of inhibition of specific [^{3}H] GBR-12935 binding to CHAPS solubilized membranes by a series of drugs was highly correlated to their relative potencies in inhibiting dopamine uptake (r=.91). Scatchard analysis of specific [^{3}H] GBR-12935 binding to CHAPS solubilized membranes revealed an apparent Kd of 10nM and Bmax of 9.1 pmole/mg protein. Partial purification of the solubilized [^{3}H] GBR-12935 binding site was achieved using a WGA lectin column. Affinity chromatography using several ligand coupled affinity gels is currently underway and the results will be presented.

517.4

CIRCADIAN RHYTHMS OF EPINEPHRINE, NOREPINEPHRINE, AND DOPAMINE IN RHESUS MONKEY CEREBROSPINAL FLUID. N.A. Garrick, I.N. Mefford,* J.L. Hill,* and D.L. Murphy,* Laboratory of Clinical Science, National Institute of Mental Health, Bethesda, MD 20892.

Diurnal variations of cerebrospinal fluid (CSF) norepinephrine (NE) and of several catecholamine metabolites have been reported in some but not all previous investigations. In the present study, CSF was collected by continuous sampling from cannulae placed in the high cervical subarachnoid space of eight chair-adapted rhesus monkeys maintained on a 12:12 light-dark cycle (lights on from 0700-1900 h). CSF epinephrine (E), NE, and dopamine (DA) were assayed by high performance liquid chromatography with electrochemical detection in the same 90-min aliquots collected over 24-h periods. E and NE exhibited clear rhythms that closely fit sinusoidal circadian models, with adjusted r^2 values of 0.83 and 0.79, respectively. Model peak to trough variations of 2.3- and 1.3-fold were found for E and NE, with peak times occurring at approximately 1400 h and 1030 h, respectively. The DA peak to trough difference was 1.65-fold with a less clearly defined rhythm (adjusted r^2 = 0.31) exhibiting a peak at 0800 h. Mean 24 h concentrations of E, NE, and DA were estimated to be 0.05 ± 0.01, 0.72 ± 0.08, and 0.22 ± 0.04 pmol/ml, respectively. While the source of E and the factors regulating its release into CSF need further study, this is the first investigation showing a marked circadian rhythm of E in primate CSF.

517.5

THE EFFECTS OF ACUTE AND CHRONIC NICOTINE ON DOPAMINE SYNTHESIS IN THE NUCLEUS ACCUMBENS, STRIATUM AND PREFRONTAL CORTEX. I.L. Holt and T.C. Westfall (Spon: J.P. Koepke). Dept. of Pharmacol. St. Louis Univ. Med. Ctr. St. Louis, MO 63104.

We have previously observed that nicotine releases dopamine (DA) from rat striatum. In the present studies we have examined the effect of nicotine on DA synthesis evaluated by measuring DOPA accumulation following inhibition of DOPA decarboxylase with NSD-1015 (100 mg/kg for 30 min). Tissues were homogenized and DOPA levels were assayed by HPLC. Basal DA synthesis was greatest in the nucleus accumbens (NAc) followed by the striatum and least in the prefrontal cortex (PFX). Acute nicotine (NIC) (1 mg/kg s.c. 45 min. prior to sacrifice) increased DOPA accumulation in the NAc (33.20± 0.72 vs 24.23± 0.38 ng/mg protein/30 min) and decreased DOPA levels in the striatum and PFX (10.31± 0.29 vs 19.45± 0.38 and 2.45± 0.21 vs 2.99± 0.08, respectively) compared to control values. Chronic NIC (1 mg/kg/day for 14 days, s.c. implanted pellet) caused a decrease in DA synthesis in the NAc, the striatum and in the PFX. DOPA levels following chronic exposure were: 19.41± 0.27 vs 24.23± 0.38 in the NAc; 16.85± 0.29 vs 19.45± 0.38 in the striatum; and 2.26± 0.03 vs 2.99± 0.08 in the PFX. These results suggest that the acute administration of NIC affects synthesis of DA by different mechanisms in the NAc and striatum and or the PFX. NIC chronically administered appears to decrease DA synthesis in all brain regions tested. (Supported by DA02668).

517.6

EFFECT OF CHRONIC NICOTINE ON THE NICOTINE INDUCED RELEASE OF ENDOGENOUS DOPAMINE IN FREELY MOVING UNANESTHETIZED RATS USING MICRODIALYSIS. T.C. Westfall and L. Vickery*. Department of Pharmacology, St. Louis University School of Medicine, St. Louis, MO 63104.

We have previously observed that the administration of nicotine (NIC) into a push-pull cannula placed in the striatum of freely moving unanesthetized rats released ^{3}H-dopamine (DA), newly synthesized from ^{3}H-tyrosine. We also observed that chronic exposure of the rats to NIC (1 mg/kg/day for 14 days by s.c. implants) resulted in an enhancement of the release of newly synthesized DA from the striatum upon rechallenge with NIC (1 µM). In the present study we utilized a microdialysis probe implanted into the striatum. Rats were anesthetized with ketamine/acepromazine and a guide cannula stereotaxically implanted (A+1.0, L+3.0, V-6.0 with respect to bregma). After 5-7 days a microdialysis probe was introduced into the guide cannula and perfused with Ringers solution (2 µl/min). Fractions were collected at 20 min intervals for analysis of DA and metabolites by HPLC-EC detection. NIC (1 mM) added to the perfusate 120 min after the start of perfusion enhanced DA release (basal 20.7±4.4; NIC 129±11.6 pg/20 min). Chronic treatment of rats with NIC (1 mg/kg/day for 14 days, s.c. implants) resulted in a significant increase in basal DA release (55±8.6 pg/20 min) while further release to NIC challenge was similar in untreated and chronically treated animals. (Supported by DA 02668.)

517.7

ACTIVATION OF TYROSINE HYDROXYLASE BY NICOTINE IN RAT ADRENAL MEDULLA. A.W. Tank and L.H. Fossom*. Univ. of Rochester Med. Ctr., Rochester, NY 14642.

Tyrosine hydroxylase (TH) activity increases rapidly in the adrenal medulla of rats that are acutely stressed. Stimulation of nicotinic receptors on adrenal chromaffin cells is thought to be a necessary step in this stress-related activation of adrenal TH. In the present study the effects of acute and chronic treatments of rats with nicotine on adrenal TH activity have been investigated. A single subcutaneous injection of nicotine activates adrenal TH in a dose-dependent manner; 2.3 mg/kg nicotine elicits a 2-3-fold increase in TH activity. This activation is maximal 15 min after the injection of nicotine; TH activity reapproaches baseline levels 45 min after the injection. Pretreatment of the animals with hexamethonium (15 mg/kg, ip) blocks the nicotine-mediated activation of adrenal TH. When rats are injected once every 30 min for 2.5 hr with 2.3 mg/kg nicotine, adrenal TH activity remains elevated persistently 3-4-fold over controls. When rats are infused chronically for 3 days with 16 mg/kg/day nicotine via Alzet osmotic pumps, a persistent 2-fold increase in adrenal TH activity is observed. A single injection of 2.3 mg/kg nicotine in these chronically-infused rats elicits a further 2-fold activation of the enzyme. Our results indicate that the intake of nicotine rapidly activates TH in the rat adrenal medulla and that tolerance to this activation does not develop in animals chronically administered the drug. (Supported by AHA, NY Affiliate Grants 87-021G.)

517.8

EFFECTS OF BIOFLAVONOIDS ON CATECHOLAMINE BIOSYNTHESIS IN CULTURED ADRENAL CHROMAFFIN CELLS. K. Morita*, H. Houchi, M. Yoshizumi* and M. Oka*. Dept. of Pharmacol., Tokushima Univ. Sch. of Med. Tokushima 770, Japan.

As a preliminary study of pharmacological actions of flavonoids on sympathoadrenergic systems, the effects of various flavonoids on catecholamine synthesis were first studied using cultured bovine adrenal chromaffin cells. The formation of [^{14}C]catecholamines from [^{14}C]tyrosine was inhibited by quercetin, but not affected by eithr apigenin or flavone. Under the conditions in which the inhibitory action on catecholamine formation was observed, quercetin failed to cause any significant effect on the uptake of [^{14}C]tyrosine into the cells. It thus seemed reasonable to assume that the inhibition of [^{14}C]catecholamine formation observed here was probably attributed to the inhibitory action of quercetin on the process of catecholamine biosynthesis rather than that on the precursor supply. The effect of quercetin on tyrosine hydroxylase activity was then examined using the enzyme prepared from bovine adrenal medulla. Quercetin caused a marked decrease in the enzyme activity, and this inhibitory action was observed in a manner dependent on its concentration. In contrast, neither apigenin nor flavone caused any effect on the enzyme. These results suggest that quercetin causes the inhibitory action on catecholamine biosynthesis through its direct action on the rate-limiting enzyme.

517.9

STIMULATORY EFFECT OF ATRIAL AND BRAIN NATRIURETIC PEPTIDES ON THE LEVEL OF CYCLIC GMP AND TYROSINE HYDROXYLASE ACTIVITY IN CULTURED ADRENAL MEDULLARY CELLS. N.Yanagihara, M.Okazaki*, T.Terao*, Y.Koda* and F.Izumi. Depts. of Pharmacology and Psychiatry, and 2nd Dept. of Int. Medicine, Univ. of Occup. & Environ. Health, School of Medicine, Kitakyushu 807, Japan.

We examined the effects of atrial natriuretic peptide (ANP) and brain natriuretic peptide (BNP) on the level of cyclic GMP and catecholamine secretion and synthesis in cultured bovine adrenal medullary cells. 1) ANP and BNP caused a significant increase in cyclic GMP level at 10^{-8}M and produced a great increase (50-100 fold) at 10^{-6}M. 2) 12-O-Tetradecanoylphorbol-13-acetate (TPA), an activator of protein kinase C, inhibited the BNP-stimulated production of cyclic GMP in a concentration-dependent manner. The half-maximal inhibitory concentration of TPA was 6.3 X 10^{-9}M. 3) Although ANP and BNP did not affect basal and carbachol-stimulated secretion of catecholamines, they caused a small but significant increase in phosphorylation and activity of tyrosine hydroxylase. Since cyclic GMP-dependent protein kinase is known to phosphorylate and activate tyrosine hydroxylase, the present results suggest that ANP and BNP enhance the phosphorylation and activity of tyrosine hydroxylase through the elevation of cyclic GMP. The peptides-induced increase of cyclic GMP seems to be modulated by protein kinase C.

517.10

Manganese Depletes Catecholamines and Biopterin in Cultured Bovine Adrenal Chromaffin Cells. R.J.Slepetis*, O.H.Viveros and A.J.Daniels. Dept. of Medicinal Biochemistry, The Wellcome Research Laboratories, Research Triangle Park, NC 27709.

The primary manifestation of manganese (Mn) neurotoxicity in vivo consists of a decrease in striatal dopamine (DA) levels; several mechanisms for this effect have been proposed, including direct displacement of DA from its storage sites by the metal ion. In an attempt to elucidate the mechanisms involved, bovine adrenomedullary chromaffin cells in culture were used as a model adrenergic neuron. The cells were incubated with 1-5mM Mn chloride, and their catecholamine content determined over time. Studies with $^{54}MnCl_2$ indicate that the uptake of Mn into chromaffin cells is temperature-dependent and essentially complete within 5 min. Upon exposure to Mn for up to 72 hours, significant decreases were observed for norepinephrine (NE), epinephrine (E), and DA. After 24 hours of incubation with Mn, cellular NE content had decreased by 32%; at 72 hours, NE levels were down by 67%. The depletion of E was less pronounced; following 24 hours of Mn exposure, it dropped by 12%, and at 72 hours, by 35%. Chromaffin cell DA levels were decreased by 72% within 24 hours, and 97.5% at 72 hours. 5mM Mn elicited a rapid drop in total cellular biopterin content; within 1 hour biopterin levels had decreased by 30%, and by 72 hours had dropped 96%. Since L-erythro-tetrahydrobiopterin is the cofactor for tyrosine hydroxylase, the rate-limiting enzyme in DA synthesis, and has been shown to be present in bovine chromaffin cells in subsaturating quantities, a substantial loss of cellular biopterin would compromise the cells' ability to synthesize new DA. Thus, catecholamine losses following Mn exposure would be due to: 1) direct displacement from storage vesicles (short-term), and 2) an inhibition of DA synthesis (long-term).

517.11

IDENTIFICATION OF THE SITES ON TYROSINE HYDROXYLASE THAT ARE PHOSPHORYLATED IN INTACT PC12 CELLS. John W. Haycock. Department of Biochemistry & Molecular Biology, Louisiana State University Medical Center, New Orleans, LA 70119

Tyrosine hydroxylase (TH), the rate-limiting enzyme in catecholamine biosynthesis, is phosphorylated at multiple sites. *In vitro*, TH purified from rat pheochromocytoma has been shown to be phosphorylated at serines 8, 19, and 40. *In situ* and *in vivo*, however, five tryptic phosphopeptides have been separated from TH phosphorylated in PC12 cells and rat corpus striatum (Neurosci. Abstr. (1988) 14:1093). In the present studies, the tryptic phosphopeptides from TH phosphorylated in intact PC12 cells were subjected to microsequencing analysis.

PC12 cells were harvested and incubated in ^{32}P-containing buffer prior to combined treatment with veratridine, forskolin and phorbol dibutyrate. The cells were sonicated, and TH was partially purified from the high-speed supernatant by heparin-Sepharose chromatography. The eluate was subjected to SDS-PAGE and electrophoretic transfer to nitrocellulose. Excised ^{32}P-TH bands were digested with trypsin, and the five, limit-digest phosphopeptides (PC1 - PC5) were separated by reversed-phase HPLC and analyzed with a Model 477A (ABI) pulsed-liquid sequenator. PC1 and PC2, phosphorylated by secretagogues, contain $serine_{19}$. PC5, phosphorylated by forskolin, contains $serine_{40}$. PC-3, shown by others to be phosphorylated by NGF, contains $serine_{153}$. PC-4, shown by others to be phosphorylated by EGF, sequenced poorly but appears to contain $serine_8$.

In vitro, $serine_{19}$ is phosphorylated by calcium/calmodulin-dependent protein kinase II (CAMPK-II); $serine_{40}$ can be phosphorylated by at least 4 different protein kinases; and $serine_8$ is phosphorylated by an unknown protein kinase that copurifies with TH. The present data are consonant with the hypothesis that CAMPK-II mediates the effects of secretagogues on TH phosphorylation.

517.12

EXPRESSION OF ENZYMATICALLY ACTIVE PHENYLETHANOLAMINE N-METHYLTRANSFERASE (PNMT) IN A MOUSE FIBROBLAST CELL LINE H. Samanta*, H. Baker, T. Wessel, T.H. Joh and D.H. Park. Lab. Mol. Neurobiol., Cornell Univ. Med. Coll., Burke Rehab. Center, White Plains, NY 10605.

Bovine adrenal medullary PNMT, the enzyme catalyzing the conversion of norepinehprine to epinephrine, has been purified and characterized biochemically, immunochemically and molecular biologically. In the present study we sought to determine biochemical and immunochemical characteristics of recombinant bovine PNMT. Bovine PNMT cDNA inserted in an expression vector was used to transfect a c127 mouse fibroblast cell line. Recombinant bovine PNMT is enzymatically active (the specific activity is 4.2 nmol product formed/mg protein/15 min, 37°C.) and is activated by phosphate in a concentration-dependent manner, reaching a plateau of 4.5 fold increase over control at 100 mM phosphate. Antibodies directed against bovine adrenal PNMT inhibit the activity of recombinant bovine PNMT and also stain immunohistochemically cells and processes in the cultures. Recombinant bovine PNMT has four charge isozymes (PI = 5.27, 5.07, 5.51 and 6.21 in decreasing order of relative abundance). Only the two minor charge forms are common between the recombinant and native enzymes. However, the existence of some variation in the charge isozymes between native and recombinant enzymes may result from differences in post-translational modification produced by the host cell. Our results suggest that recombinant PNMT, expressed from bovine cDNA in a mouse fibroblast cell line, is enzymatically active and shares many common features with native bovine adrenal enzyme.
Supported by grant # MH 44043.

517.13

CHARACTERIZATION OF D1 DOPAMINE RECEPTORS IN THE BOVINE PINEAL GLAND. V. Simonneaux*, L.C. Murrin and M. Ebadi. Dept. of Pharmacology, Univ. of Neb. College of Medicine. 42nd and Dewey Ave., Omaha, NE 68105

Previous studies from our laboratory have shown that bovine pineal glands contain a high concentration of dopamine (4.0 μg/mg tissue) and dopamine D2 receptors. Dopamine exhibited dual effects on the activity of serotonin N-acetyltransferase, inhibiting the basal activity at 0.1 μM and stimulating it at 10 μM. In this study we examined dopamine D1 receptors in pineal gland. The assays were carried out in 2 ml of 0.05 M Tris buffer plus ions (ph 7.4) containing 150 μg protein and 0.4 nM [^{3}H]SCH 23390 (72.5 Ci/mmole) as ligand. Incubations were for 15 min at 37°C and were terminated by vacuum filtration followed by three 5 ml washes with ice-cold buffer. In association rate studies, equilibrium was reached within 10 min with 0.4 or 0.04 nM [^{3}H]SCH 23390 and remained stable for 30 min. The association rate constant (k_1) was calculated to be 0.65 nM^{-1} min^{-1}. Dissociation of [^{3}H]SCH 23390 was rapid with a $t_{½}$ of 4 min and a calculated dissociation rate (k_{-1}) of 0.15 min^{-1}. Kinetic studies gave an estimated K_D value of 0.23 nM. Saturation studies (using 0.013 to 3.50 nM [^{3}H]SCH 23390) indicated the presence of only one binding site with a K_D value of 0.57 ± 0.05 nM and a receptor density of 974 ± 41 fmol/mg protein, which is about 30 times the density of D2 receptors in pineal. Competition binding studies with a variety of agents active at dopamine and serotonin receptors indicated that the majority of the binding was due to D1 dopamine receptors. The results of this study indicate that the functions of dopamine in the pineal gland are modulated through mechanisms which involve both D1 and D2 dopamine receptors. This study was supported in part from the grants Simone and Cino del Duca (V.S.), NS23975 (L.C.M.) and ES03949 (M.E.).

517.14

EFFECTS OF INHIBITORS OF EPINEPHRINE (E) FORMATION ON PINEAL E CONTENT. J. Opacka-Juffry*, M.C. Ruiz de Elvira* and C.W. Coen. Dept. Anatomy & Human Biology, King's College London, U.K.

Estimation of norepinephrine (NE) and E turnover in the pineal gland of female Wistar rats was attempted using FLA 63, an inhibitor of dopamine ß-hydroxylase. Paradoxically, 10 mg FLA 63/kg i.p. resulted in a significant increase (+77%) in pineal E content within 2 hours without affecting that of NE. In contrast, in the median eminence (ME) and hypothalamic preoptic area (POA) this treatment caused a depletion of both NE and E (range -36% to -72%). Another blocker of E synthesis, LY 134046, an inhibitor of phenylethanolamine N-methyltransferase (PNMT) (40 mg/kg i.p., 5 and 2 hours before sacrifice) caused an increase (+224%) in pineal E content with no effect on NE. The same treatment resulted in the depletion of E in the ME and POA (-50% and -71%, respectively). The increase in pineal E content following LY 134046 was prevented by prior adrenalectomy. This suggests that adrenal medulla is the primary source of increased pineal E and that circulating E is taken up by the pineal and possibly stored in the terminals of sympathetic nerves innervating this gland. These effects on the pineal E following inhibitors of E formation resemble those following immobilization (Saavedra J.M. et al., Cell. Mol. Neurobiol., 2:1, 1982). The biological implications of this phenomenon remain to be elucidated. [Supported by MRC G8220475 N.]

517.15

REGULATION OF DOPAMINE SYNTHESIS IN VIVO BY SELECTIVE 5HT-1 RECEPTOR AGONISTS. P.A. Johansen and M.P. Galloway, NPRU, Lafayette Clinic, Cell. & Clin. Neurobiol., Dept. of Psychiatry, Wayne State Univ. Sch. Med., Detroit, MI.

Given recent evidence for the potential involvement of 5-HT systems in reward, the present study examined the effect of a 5-HT-1A agonist, 8-OHDPAT, and a 5-HT-1B agonist, TFMPP, on dopamine (DA) synthesis in vivo. DA synthesis was determined in rats as DOPA accumulation after administration of NSD-1015 (100 mg/kg, 30 min before sacrifice). Administration of TFMPP (30 µmol/kg s.c., 40-50 min prior to sacrifice) produced a three- to four-fold increase in DOPA in the nucleus accumbens (NA) and striatum (ST) and a 55% increase in prefrontal cortex (PFc), whereas 3 µmol/kg TFMPP produced only a modest increase or was ineffective. In contrast, 8-OHDPAT (30 µmol/kg) decreased DOPA accumulation in NA (-30%), and exerted no effect on DA synthesis in ST or PFc. However, coadministration of 8-OHDPAT and TFMPP appeared to attenuate the robust increase in DOPA produced by TFMPP in the NA and ST. Following pretreatment with reserpine, TFMPP did not enhance DA synthesis. In rats treated with gamma-butyrolactone (GBL), TFMPP did not reverse the GBL-induced increase in DOPA; rather, a further enhancement (60%) of DOPA (over GBL alone) was observed in the NA. In addition, TFMPP did not block the ability of quinpirole to reverse the GBL-induced increase in DOPA. Thus, 5-HT-1B receptors appear to be involved in the modulation of DA synthesis in vivo. (Support: DA 04120, MH 41227, State of Michigan DMH)

517.16

REGULATION OF DOPAMINE RELEASE BY SEROTONIN (5HT) 1A AND 1B AGONISTS MEASURED BY MICRODIALYSIS. S. Benloucif and M.P. Galloway, Lafayette Clinic, CCN Program, Dept. of Psychiatry, Wayne State Univ Sch. Med. Detroit, MI 48207

Work in our laboratory indicates that agonists with specificity for serotonin 1A and 1B receptors alter synthesis of dopamine (DA), as well as serotonin, in vivo. We have initiated a series of studies utilizing microdialysis to investigate the effects of 5HT agonists on DA release in vivo. Microdialysis probes were placed in the anterior-lateral striatum of chloral hydrate anesthetized rats and perfused with a modified Ringers solution containing the 5HT reuptake inhibitor citalopram (1 µM) at a rate of 2 µl/min. Recovery was relatively stable in the second hour following probe implantation, and 30 min samples yielded an average 0.8 pg/µl of DA and 0.15 pg/µl of 5HT. Agonists were administered at least 2 hours following probe implantation. Local administration of the 5HT-1B agonist TFMPP (1 mM in the microdialysis probe) increased extracellular DA to an average of 7 pg/µl (9 fold increase over baseline, N=3). Extracellular 5HT was not decreased under these conditions. TFMPP did not substantially affect recovery of DA or 5HT standards in vitro. Administration of the 5HT-1A agonist 8-OHDPAT (10 µM i.c. or 3 or 30 umoles/kg s.c.) did not alter DA release. These findings support the hypothesis that 5HT-1B agonists alter dopaminergic activity. (Supported by MH-41227, DA-04120, and the State of Michigan DMH).

517.17

COLD-SWIM STRESS INDUCES TIME-DEPENDENT CHANGES IN CENTRAL NEURAL PHENYETHANOLAMINE N-METHYL TRANFERASE (PNMT) AND EPINEPHRINE IN THE HYPOTHALAMUS AND MEDULLA/PONS OF THE RAT. N.C. Fernandez*, T.D. Gbadebo*, N. Narasimhachari, and J.K. Stewart. Depts. of Biology and Psychiatry, Virginia Commonwealth Univ., Richmond, VA 23284.

Male Sprague Dawley rats swam for 3 min in 5 or 37 C water and were killed 1 or 4 hr later. Swimming in cold water decreased PNMT activity 1 hr later in the hypothalamus and medulla/pons:

	UNTREATED	COLD	WARM
Hypo PNMT	1.53±0.17	0.98±0.12[a]	1.75±0.25
(N)	(5)	(6)	(4)
Med PNMT	2.59±0.22	1.98±0.31[b]	3.25±0.41
(N)	(9)	(9)	(9)

Values are the mean±SE pmol/(hr x mg protein)
[a]P<0.05 compared to the untreated or warm-swim group.
[b]P<0.05 compared to the warm-swim group.

Four hr after cold swim stress PNMT activity in the medulla/pons was 25% greater than in untreated animals, and in the hypothalamus enzymatic activity had returned to control levels. Despite the recovery of hypothalamic PNMT, epinephrine levels in the hypothalamus were lower 4 hr after cold-swim stress than in untreated rats ($P<0.05$). These findings support the hypothesis that hypothermia inhibits epinephrine synthesis in the brainstem and hypothalamus.

517.18

EFFECT OF ACUTE AND DAILY COCAINE AND NEUROTENSIN ON EXTRACELLULAR DOPAMINE IN THE ACCUMBENS AND A10 REGION. P.W. Kalivas and P. Duffy. Dept. of VCAPP, Washington State University, Pullman, WA 99164-6520.

Acute peripheral injection of cocaine, and microinjection of neurotensin (NT) into the A10 dopamine (DA) region produces an increase in motor activity in rodents. After daily administration of either drug the behavioral response is augmented. Enhanced DA transmission in the accumbens (NA) has been implicated in these effects. To directly evaluate a role of DA release into the NA, a removable dialysis probe (250 um wide x 2 mm long) was inserted into the NA of conscious rats. In rats pretreated with daily cocaine (15 mg/kg, ip x 4 days) or NT (0.5 nmol/side x 4 days) an acute injection of the drug produced a significantly greater increase in extracellular DA in the NA that was observed in rats pretreated with daily saline. It has been postulated that a decrease in somatodendritic DA release in the A10 may occur after daily cocaine injection, and by inserting a dialysis probe into the A10 region, it was found that while acute cocaine elevated extracellular DA in the A10 region, after daily cocaine treatment, an acute injection no longer increased DA release. Thus, behavioral sensitization to cocaine and neurotensin is associated with an augmentation in extracellular DA in the NA, and a decrease in the A10 region.

517.19

MODULATION OF STRIATAL AROMATIC L-AMINO ACID DECARBOXYLASE. **M. Hadjiconstantinou, C.P. Sylvia*, J.P. Hubble*, L.A. Isaacs*, T.A. Wemlinger* and N.H. Neff. Departments of Psychiatry and of Pharmacology, The Ohio State University College of Medicine, Columbus, OH, 43210.**

Aromatic L-amino acid decarboxylase (AAAD) is the second enzyme in the sequence leading to the formation of catecholamines. It is not considered to be rate limiting for catecholamine formation except in parkinsonian patients under L-DOPA treatment. Recently we have reported that light as well as α-2 adrenergic and dopamine (DA) D-1 receptor antagonists increase enzyme activity in retina. We now provide evidence that AAAD activity of the striatum can be modulated by DA receptor antagonist drugs. The ability to modulate AAAD may be, in part, determined by the vitality of dopaminergic striatal nerve terminals, since we were able to increase enzyme activity in animals with striatal lesions but not in control animals. MPTP, 30 mg/kg, ip, was administered to mice for 7 days. This treatment results in about a 50% decrease of DA content and AAAD activity in the striatum. After three weeks post-lesion there is recovery of AAAD to near control values but not of DA content. Administration of the selective D-1 receptor antagonist SCH 23990 24 h after the completion of the MPTP treatment resulted in an increase of striatal AAAD activity in a dose- and time- dependent manner. SCH 23990, 5 mg/kg, produced a maximal response in about 6 h in lesioned mice but had no effect in control animals. Similarly the non-selective DA antagonist haloperidol and the selective D-2 antagonist sulpiride augmented AAAD activity in the striatum of MPTP-treated mice. Again, these compounds had no effect in untreated mice. Administration of a single dose of the selective D-1 agonist SKF 38393 or the selective D-2 agonist quinpirole had no effect on enzyme activity in both control and MPTP-treated mice. However, pretreatment with SKF 38393 prevented the SCH 23990-induced increase of AAAD activity in the MPTP-treated animals. These observations may have significant implications for parkinsonians being treated with L-DOPA alone or together with a DA agonist.

517.20

DIFFERENTIAL TETRAHYDROBIOPTERIN METABOLISM IN CENTRAL AND PERIPHERAL MONOAMINE NEURONS. G. Kapatos, H. Hasegawa, K. Hirayama, N. Relan and V. Kemski. Center for Cell Biology, Sinai Research Institute, Sinai Hospital of Detroit and Cellular and Clinical Neurobiology, Program, Wayne State University, Detroit, MI. 48235.

Although tetrahydrobiopterin (BH4) is limiting in the synthesis of monoamines, virtually nothing is known regarding the synthesis and degradation of this essential cofactor. In addition, clinical observations suggest that BH4 metabolism may be different in central and peripheral monoaminergic neurons. Monolayer cultures of mesencephalic (MES) dopamine-containing (DA) neurons derived from embryonic day 15 rat brain and noradrenergic (NE) sympathetic (SYM) neurons derived from the neonatal rat superior cervical ganglia were used as models of central and peripheral monoamine neurons, respectively. Cultures were incubated with either N-acety1-serotonin (NAS, 200uM) or 2,4-diamino-6-hydroxypyrimidine (DHP, 10mM), compounds which inhibit BH4 biosynthesis by different mechanisms. Cultures were harvested at 1-8 hours and BH4 levels determined. Both NAS and DHP produced identical, and rapid declines in MES BH4 levels, exhibiting a t1/2 of 3 hours. In contrast, while DHP decreased BH4 levels in SYM neurons, with a t1/2 of 3 hours, NAS was without effect. These data suggest that BH4 metabolism is much more rapid than expected, and may be different within central DA and peripheral NE neurons. (supported by NS-26081)

518.1

REPEATED STIMULATION OF D1 DOPAMINE RECEPTORS ENHANCES GROOMING AND NEURONAL RESPONSES TO SKF-38393 (SKF) F.J. White, R.J. Brooderson, and X.-T. Hu, Dept. Psychiatry, Neuropsychopharmacol. Lab., Wayne St. Univ. Sch. Med., Lafayette Clinic, Detroit, MI 48207

Behavioral observations and extracellular single unit recording techniques were employed to examine rat grooming and neuronal responses of caudate-putamen (CPu) cells, respectively, to the selective D1 agonist SKF following 3 weeks pretreatment (8mg/kg, twice/day, s.c.) with the same compound. After 1 week drug withdrawal, the amount of time in which rats engaged in grooming following SKF (8mg/kg) challenge was significantly prolonged as compared to either untreated or saline-treated rats. Consistent with the behavioral observations, the inhibitory responses of CPu cells to microiontophoretic administration of SKF were enhanced in SKF-treated rats when compared to controls. The repeated SKF pretreatment also enhanced the inhibitory responses of CPu cells induced by the selective D2 agonist quinpirole. However, neither behavioral nor electrophysiological supersensitivities were observed after chronic SKF treatment without 1 week drug withdrawal. These results suggest that, with a period of drug withdrawal, chronic stimulation of D1 receptors with SKF may result in D1 receptor supersensitivity which enhances the enabling effect of D1 receptors on D2 responses (Supported by APDA, USPHS Grants DA-04093, and MH-40832 to FJW).

518.2

THE EFFECT OF NOREPINEPHRINE ON ENDOTOXIN-MEDIATED MACROPHAGE ACTIVATION. Xiaoxi Hu* and Celia F.Brosnan* (SPON:M Litwak). Dept Pathology, Albert Einstein Coll Med, Bronx, NY 10461.

Recent studies have suggested that sympathetic innervation of the spleen may directly contact cells of the immune system and thus might alter the course of an immune response (Felten et al, Brain Res Bull 6:83, 1981). To investigate this possibility, macrophages were isolated from rat spleen by adhesion to plastic and incubated with lipopolysaccharide (10 ug/ml) for 18h in the presence of varying doses of norepinephrine (NE). Secreted and membrane -bound samples were then tested for lymphocyte activating factors (LAF) using [3H]-thymidine incorporation into PHA (lug/ml) stimulated mouse thymocytes, and for tumor necrosis factor (TNF) activity using L929 cells. The results show that both secreted and cell associated LAF and TNF activity are diminished in NE-treated cells. Dose response curves demonstrated a bimodal curve with suppression noted at 10^{-5}, 10^{-6} and 10^{-8}, 10^{-9}M. NE was more potent than epinephrine and the effect could be partially reversed with propranolol but not with phentolamine, indicating the presence of a beta-receptor. Since factors released by macrophages initiate many aspects of the immune response these results support a functional role for sympathetic innervation of the spleen in immune regulation. Supported by NS 23247 and NMSS 1089

518.3

DIETARY CALCIUM ALTERS REACTIVITY TO NOREPINEPHRINE. D.C. Hatton*, K.S. Scrogin*, D. Martinsen*, D.A. McCarron* (Sponsor M. Meikle). Dept. Med Psy. & Div. of Nephrol. Ore. Health Sci. Univ., Portland, OR 97201 and Dept. Biol., Lewis & Clark College, Portland, OR 97219

One week of exposure to supplemental dietary calcium lowers blood pressure in weanling SHRs. The mechanisms responsible for the alteration in blood pressure have not been identified. This study investigates the effects of dietary calcium on plasma catecholamines and blood pressure reactivity to bolus infusions of norepinephrine. Twenty-one day old SHRs were maintained on either a high (2.0%) or low (0.1%) calcium diet for 7 days. On the eighth day, direct arterial blood pressure was recorded followed by a 0.5 ml blood sample withdrawal or by bolus infusions of 5, 10, and 20 ug/kg norepinephrine (NE). The calcium deficient SHRs had significantly higher blood pressures than SHRs fed high calcium diets (117 mmHg vs 105 mmHg, $p<.05$). Plasma NE was higher in the animals on low calcium diets but the difference did not reach statistical significance (480 pg/ml low diet vs 369 pg/ml high diet, $p=.07$). There were significant differences in blood pressure reactivity to bolus infusions of NE ($p<.001$) between the two diet groups with animals in the low diet group having more prolonged pressor episodes at all three dose levels. The results indicate that differences in vascular responsivness to NE, or differences in NE uptake, may be responsible for the observed differences in blood pressure.

518.4

EFFECTS OF DIETARY TYROSINE AND OTHER AMINO ACIDS ON HEMODYNAMIC RESPONSES AND PLASMA AND TISSUE TYROSINE LEVELS. M.S. Eisenberg* and T.J. Maher. Department of Pharmacology, Massachusetts College of Pharmacy, Boston, MA 02115.

In catecholamine(CA)-containing neurons made to fire rapidly for prolonged periods, maintained CA synthesis and release is believed to be dependent on tyrosine(TYR) availability. Sympathetic reflexes become activated in rats subjected to a continuous(0.2ml/min) hemmorhage(HEM) and in response to 20ug/kg i.v. hydralazine(HDZ). In these studies dietary TYR(4X the normal amount) maintained blood pressure (BP) in HDZ-induced hypotension and BP and heart rate(HR) during HEM. Diets high(4X normal) in phenylalanine(PHE), alanine(ALA) and valine(VAL), as well as 8X normal TYR failed to maintain BP or HR during severe HEM. The effect of dietary amino acid supplementation on plasma(PL), peripheral and brain tissue stores of TYR after a 5-day feeding period was also tested. PL TYR significantly increased by 54% and 779% above control levels in the 4X and 8X TYR, respectively. Both TYR diets significantly elevated heart, adrenal, kidney, spleen, brainstem and cortex TYR; while only 8X TYR increased liver TYR. PHE significantly elevated TYR only in PL and brain. ALA, which increases BP during an acute but not severe HEM, elevated heart and adrenal TYR. VAL elevated adrenal TYR and significantly decreased brainstem and cortical TYR. Thus, dietary TYR increases TYR stores in PL and tissues and may be utilized when CA synthesis depends on additional TYR, such as in hypovolemic- or HDZ-induced HEM.

518.5

METABOLISM OF L-THREO-3,4-DIHYDROXYPHENYLSERINE (L-THREO-DOPS) IN MOUSE BRAIN; IN VITRO AND IN VIVO STUDIES. A.B.Naini*, L.J. Cote and F.Arnal* (SPON: E.Housepian), Dept of Neurology, Coll. of P&S, Columbia Univ, New York, N.Y. 10032.

L-threo DOPS is the stereoisomer of DOPS that, when decarboxylated, yields the biologically active (-)NE. In the in vitro studies, whole mouse brains were homogenized (1:10) in ice cold 0.6 M phosphate buffer (pH 7.4). Aliquots (1 ml) of the homogenate were incubated at 37°C for 20 min in the presence of 68µM L-threo-DOPS, and in some experiments 50µM pyridoxal phosphate (Pyr P) was added. The NE formed was extracted on alumina and quantitated using HPLC with EC detection. The NE level rose by 35% in the absence of Pyr P, but when Pyr P was added no such increase in NE occurred. The addition of Pyr P to the homogenate in the absence of L-threo-DOPS did not affect the level of NE in the homogenate.

In the in vivo studies, mice were given L-threo-DOPS (100 mg/kg) I.P. and sacrificed at 30, 60 and 120 min after injection. The brains were homogenized and assayed for DOPS, NE and MHPG. DOPS in the brain reached the maximum level at 1 hr after injection (1102±156 ng/g w.wt.); at 2 hrs DOPS dropped to 615±120 ng/g w.wt. NE and MHPG rose by 19% and 100%, respectively, at 30 min after injection and remained elevated up to 2 hrs. Mice given Pyr P (10 mg/kg) I.P., as well as L-threo-DOPS (100 mg/kg) failed to show an increase in the brain NE or in MHPG.

In conclusion, L-threo-DOPS is decarboxylated to NE both in vitro and in vivo, however, the presence of Pyr P interferes with the reaction, possibly due to a complex formed between L-threo-DOPS and Pyr P.

518.6

THE D2 AGONIST QUINPIROLE INDUCES NON-DIRECTED AND DIRECTED CHEWING IN WEANLING RATS. C.A. Moody and L.P. Spear. Dept. of Psychology and Centers for Developmental Psychobiology and Neurobehavioral Science, SUNY, Binghamton NY 13901

Weanling (21 day old) Sprague-Dawley rat pups were s.c. injected with 0 (saline), 0.05, 0.1, 0.5 or 1.0 mg/kg/3cc of the D2 agonist quinpirole and individually placed in a test apparatus containing a dish of wet mash and either a food pellet or wood block. Behaviors were recorded for 5 min. via time-sampling at 30 and 60 min. post-injection. The 3 highest doses of quinpirole increased the amount of wall climbing/supported rear, probing, sniffing, and forward locomotion. These doses of quinpirole also induced both non-directed chewing behavior (mouthing) as well as chewing directed at both the wood block and the food pellet suggesting that this chewing was not strongly nutritive in nature. Although in adult animals quinpirole has been reported to induce dose-dependent increases in locomotion, sniffing and rearing, oral behaviors in adults are not induced by quinpirole alone (Arnt et al., 1987), and indeed have been reported to be inhibited by quinpirole treatment (Ellison et al., 1988).
Supported by DA04478 to LPS.

518.7

D-1 DOPAMINE (DA) RECEPTOR STIMULATION ACTIVATES GLYCOGEN PHOSPHORYLASE IN RAT STRIATAL SLICES. L.D. Kreamer*, C.F. Saller and A.I. Salama (SPON: B. Dubinsky). Dept. of Pharmacology, ICI Pharmaceuticals Group, Wilmington, DE 19897.

D-1 DA receptor stimulation increases cAMP formation. Since glycogen phosphorylase is activated via a cAMP-dependent process, the possible role of D-1 receptor stimulation in glycogen phosphorylase activation was examined in rat striatal tissue slices. Incubation of tissue with a selective D-1 agonist, SKF 38393 (50 and 100μM), activated glycogen phosphorylase. The SKF 38393-induced activation was prevented by pre-incubation with the D-1 selective antagonist, SCH 23390 (10μM). By itself, SCH 23390 had either no effect or slightly decreased striatal glycogen phosphorylase activity. Therefore, D-1 receptor stimulation appears to activate glycogen phosphorylase in vitro, indicating a possible role for DA in the regulation of brain carbohydrate metabolism. In fact, preliminary in vivo studies provide some support for this possibility. However, the data obtained thus far, while often significant, have been variable, suggesting that other factors such as endogenous dopaminergic activity or stress might be important factors in vivo.

518.8

DOPAMINE (DA) D-2 RECEPTOR STIMULATION IN BRAIN INCREASES BLOOD GLUCOSE CONCENTRATIONS. C.F. Saller, L.D. Kreamer* and A.I. Salama. Dept. of Pharmacology, ICI Pharmaceuticals Group, ICI Americas Inc., Wilmington, DE 19897.

The D-2 DA receptor agonists quinpirole and N-0434 increased blood glucose. These increases were prevented by centrally acting D-2 receptor antagonists. For example, quinpirole (1 mg/kg,i.p.) or N-0434 (5 mg/kg, i.p.), given to rats 20 min. before decapitation, increased ($P<0.05$) blood glucose by 57% and 38%, respectively. Pretreatment with 1 mg/kg,i.p. of either eticlopride or haloperidol, D-2 antagonists, 20 min. before the agonists, prevented the D-2 agonist-induced increases in blood glucose. Alone, neither antagonist altered blood glucose. Likewise, domperidone (2.5 mg/kg,i.p.), a D-2 antagonist which does not readily enter the brain, did not alter blood glucose Pretreatment with domperidone also failed to prevent quinpirole-induced increases in blood glucose, indicating that central D-2 receptors mediate this effect. Not unexpectedly, the increases in blood glucose produced by quinpirole were accompanied by increases in brain glucose. For example, quinpirole (0.25 mg/kg,i.p.) increased ($P<0.05$) blood and striatal glucose by 36% and 44%, respectively. Neither SKF 38393, a selective D-1 agonist, nor SCH 23390, a selective D-1 antagonist, altered blood glucose. Thus, these observations suggest that the stimulation of D-2 receptors in brain can increase blood glucose.

518.9

ALPHA-1 ADRENERGIC MEDIATION OF NORADRENALINE-STIMULATED GLYCOGENOLYSIS IN THE RAT OLFACTORY BULB. M. Hussong, R. Coopersmith, and M. Leon. Dept. of Psychobiology, Univ. of California, Irvine, CA 92717.

The rat olfactory bulb has very high levels of glycogen phosphorylase, the enzyme which breaks down glycogen to glucose (Coopersmith, R. and Leon, M., J. Comp. Neurol., 261:148, 1987). Noradrenaline is a potent glycogenolytic agent in olfactory bulb slices, capable of activating phosphorylase at concentrations three orders of magnitude lower than the dose required in mouse cortical slices. We now report that phenylephrine, an alpha-1 adrenoceptor agonist, is as potent a glycogenolytic agent in olfactory bulb slices as is noradrenaline.

Olfactory bulb slices were incubated with tritiated glucose for 30 min, to label glycogen. Phenylephrine, at doses ranging from 1 pM to 10 μM was then added to the slices. After 25 min, slices were homogenized, radioactive glucose and amino acids were removed, and the radioactivity remaining in glycogen was determined. Phenylephrine induced a concentration-dependent breakdown of glycogen. The maximal effect was a 55% loss of label, the same as seen with noradrenaline. The EC50 was approximately 100 pM, slightly lower than that for noradrenaline. Clonidine, an alpha-2 agonist, was ineffective at a dose of 10 nM. In cortex, noradrenaline-stimulated glycogenolysis is mediated by beta-adrenoceptors, and alpha agonists have little effect. The contrasting results in olfactory bulb are consistent with the high density of alpha-adrenoceptors found in this structure.

518.10

EFFECT OF APOMORPHINE ON OXYTOCIN CONCENTRATION IN RAT BRAIN AND PLASMA. M.R. Melis and A. Argiolas. Department of Neurosciences, University of Cagliari, Via Porcell 4, 09124 Cagliari (Italy).

The effect of the systemic injection of the dopamine agonist apomorphine (APO) on the concentration of oxytocin in plasma and different brain areas was studied in male rats by a specific radioimmunoassay. In plasma APO given s.c. in doses ranging from 80 to 480 g/kg increased oxytocin levels in a dose dependent manner. The minimal effective dose was found to be 80 g/kg which induced a 66% increase above basal values while the maximal effect (210%) was seen with the dose of 240 g/kg. APO effect was prevented by the DA-D2 receptor blockers haloperidol (0.2 mg/kg i.p.) or (-)sulpiride (10 mg/kg i.p.) and, but only partially, the DA-D1 receptor blocker SCH 23390 (0.2 mg/kg s.c.). On the other hand, the above doses of APO induced a small decrease and a dose-dependent increase in oxytocin concentration in the hypothalamus and the hippocampus, respectively. As found in plasma haloperidol and sulpiride prevented APO-induced changes. In contrast, no change was found in septal oxytocin. The present results suggest that dopamine has a facilitatory role not only on oxytocin relase in the periphery but also in the CNS of male rats.

518.11

CONTINUOUS AND INTERMITTENT ADMINISTRATION OF SKF 38393 OR QUINPIROLE DIFFERENTIALLY AFFECT D-1 AND D-2 AGONIST-INDUCED ROTATION. T.M. Engber, Z. Susel* and T.N. Chase. Exptl. Therapeutics Branch, NINDS, Bethesda, MD 20892.

The effects of continuous and intermittent administration of either the D-1 dopamine agonist SKF 38393 or the D-2 agonist quinpirole on rotational behavior induced by these selective agonists were compared in rats with a unilateral 6-hydroxydopamine lesion of the median forebrain bundle. Rats were divided into 3 treatment groups: 1) continuous vehicle + intermittent vehicle (control group), 2) continuous agonist + intermittent vehicle, or c) continuous vehicle + intermittent agonist. Continuous treatments were given via Alzet osmotic pumps (Model 2ML2) implanted i.p.; intermittent treatments consisted of i.p. injections given once daily. The same daily dose was administered for both continuous and intermittent agonist treatments. SKF 38393 was given at a dose of 12.5 mg/kg/day in a vehicle of 12.5% ascorbate in 50% DMSO; quinpirole was given at a dose of 1 mg/kg/day in 0.01% ascorbate in water. Following 19 days of treatment and a 3 day washout, rats (n=6 in each group) were tested for rotational response to either SKF 38393 (1.25 mg/kg, i.p.) or quinpirole (0.1 mg/kg, i.p.). Continuous SKF 38393 abolished the subsequent rotational response to SKF 38393 (0.9% of control) but had no effect on the response to quinpirole. Intermittent SKF 38393 substantially reduced the rotational response to SKF 38393 (9% of control), but increased the response to quinpirole (175% of control). Continuous quinpirole had no effect on the rotational response to either SKF 38393 or quinpirole. Intermittent quinpirole greatly enhanced the rotational response to both SKF 38393 (279% of control) and quinpirole (202% of control). These results indicate that chronic treatment with D-1 and D-2 dopamine agonists differentially affect rotational behavior mediated by D-1 and D-2 dopamine receptors and, furthermore, that continuous and intermittent treatment schedules can profoundly influence the nature of the subsequent rotational response.

518.12

STIMULATION OF D2 RECEPTORS IN RAT PREFRONTAL CORTEX INHIBITS THE ELECTRICALLY-EVOKED RELEASE OF ^{3}H-GABA J.Pénit-Soria*, S.Rétaux*, Y.Maurin*
(SPON: J.P.Bourgeois) Inst. Neurosci., Univ. P&M Curie, 75005 Paris, France

In vivo[1] and in vitro[2] studies suggest that dopaminergic neurons of the A10 area inhibit the firing of prefrontal cortex (PFC) neurons through the activation of GABAergic interneurons. We investigated the effects of D1 and D2 agonists and antagonists on the electrically-evoked release of ^{3}H-GABA in PFC slices (3ms, 24 mA, 5 Hz, 2 min). This release was largely calcium dependent. It was not affected by the D1 agonist SKF 38393 but inhibited by the two D2 agonists lisuride and RU 24926 (respective IC50's : $2x10^{-7}$M ; $4x10^{-8}$M). The D2 agonist LY 171555 remained ineffective. The effects of 10^{-7}M RU 24926 were fully reversed, and those of 10^{-6}M lisuride partially reversed by the selective D2 antagonist sulpiride 10^{-5}M. Although exogenous DA (10^{-4}M) was devoid of effect, amphetamine (10^{-5}M) inhibited ^{3}H-GABA release, and this effect was antagonized by 10^{-5}M sulpiride. Our results show that in the PFC, the activation of DA receptors with D2 properties inhibits GABA release.

1. Thierry et al., 1986, Brain Res. Bull., 16:155-160
2. Pénit-Soria et al., 1987, Brain Res., 425:263-274.

Supported by DRET grant 88/1313.

518.13

DOPAMINE IN CAT VISUAL CORTEX FOLLOWING AN ANTICHOLINESTERASE AGENT. A.T. Townsend* and A.W. Kirby (SPON: R. Pourcho). USAARL, P.O. Box 577, Ft. Rucker, AL 36362.

Administration of diisopropylfluorophosphate (DFP), an irreversible inhibitor of acetylcholinesterase (AChE), results in alteration in the cat visual evoked response (VER), and neurochemical changes in visual centers. Over 15-20 hours, the VER recovers but AChE activity does not. These experiments were done to investigate the role of dopamine (DA) in these changes.

After i.v. DFP, anesthetized and paralyzed cats show a preferential low spatial frequency loss in the VER (14 of 14) and increased DA turnover in visual cortex (13 of 14) at all survival times, even when the VER had recovered to baseline.

DFP was applied directly to the surface of the visual cortex of anesthetized cats to determine if DA turnover is locally mediated. Preliminary results show increased DA turnover in DFP cortex compared to control (opposite side, same animal). It appears that increased DA turnover in cat visual cortex is locally mediated after DFP, and may play a critical role in VER changes.

518.14

COMPUTATIONAL MODELING OF α and β NORADRENERGIC ACTIONS AT DENDRITIC VS. SOMATIC LOCI OF DENTATE GRANULE NEURONS.
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Albert Einstein College of Medicine, Bronx, NY 10461.

Norepinephrine (NE) is a modulator of neuronal plasticity in the hippocampal dentate gyrus, enhancing long-term potentiation of perforant path synapses and having direct long-lasting actions on granule cell membranes. There are multiple effects on granule neurons mediated by both α and β-receptor subtypes, with evidence for both somatic and dendritic sites of action. A balance of effects determines whether firing is enhanced or reduced, and various cell types can be differentially modulated by NE.

In light of difficulties determining somatic vs. dendritic sites of action experimentally, we mathematically modelled dentate granule neurons and simulated effects of specifically activating single α or β-receptor actions on conductances at either somatic or dendritic loci. We used a multi-compartment neuron with Hodgkin-Huxley and other active ion channels plus the Nernst-Planck equation for electrodiffusion of ions in dendritic spines. The simulator was written with structured object-oriented programming techniques for easy manipulation of physiologically relevant parameters, measuring voltage and calcium in both somatic and dendritic compartments.

Noradrenergic effects simulated included β-receptor mediated blockade of the late Ca^{2+}-dependent K^+ conductance g(K)Ca and α-receptor block of regenerative Ca^{2+} action potentials. We have found markedly different effects of somatic versus dendritic sites of NE action, suggesting that spatial distribution of noradrenergic receptors offers a sensitive way of postsynaptically altering NE regulation of signal processing and storage.

518.15

TURNOVER OF BIOGENIC AMINES IN DISCRETE AREAS IN THE RAT BRAIN AND THE SIGNIFICANCE OF L-AROMATIC AMINO ACID DECARXYLASE ACTIVITIES. A.Y-C Shum*, D-J Juang*, C-F Chen* and J-Y Wang. Department of Pharmacology, National Yang-Ming Medical College and Department of Physiology, National Defence Medical Center, Taipei, Taiwan, ROC

Accumulation of the intermediates dihydroxyphenylalanine (DOPA) and 5-hydroxytryphan (5HT) following l-aromatic amino acid decarboxylase (LAAAD) inhibition with the drug m-hydroxybenzylhydrazine (NSD1015) permits the simultaneous estimation of turnover of the neurotransmitters catecholamines (CA) and 5-hydroxytryptamine (5HT). However, the nature and significance of this inhibition remained controversial. The present experiments were designed to clarify some of these questions. *In vitro* enzymic analyses following *in vivo* administration of NSD indicated dose-related inhibition in a competitive manner. Although NSD apparently does not produce depletion of tissue levels by itself, the accumulation of these amines following inhibition of the metabolizing enzyme monoamine oxidase (MAO) was significantly reduced. Exogenous administration of DOPA and 5HTP in conjunction with MAO inhibition resulted in accumulation of 5HT and mainly dopamine (DA) in all areas studied. It is thus concluded that LAAAD was effectively and dose-dependently inhibited by NSD and that DOPA accumulation mainly represented DA turnover. The lack of effects on tissue levels of monoamines may be due to the fact that NSD also appeared to inhibit MAO.

518.16

THE EFFECTS OF STRESSOR CONTROLLABILITY ON REGIONAL CHANGES IN MESOCORTICOLIMBIC DOPAMINE ACTIVITY.

L. W. Fitzgerald*, R.W. Keller, S.D. Glick, J.N. Carlson. Dept. of Pharmacology & Toxicology, Albany Medical College, Albany, NY 12208

While it has been known for some time that stressful stimuli induce an activation of dopamine (DA) neurons projecting to the medial prefrontal cortex (PFC) and the nucleus accumbens (NA) in the rat, few studies have assessed the effects of the ability to control the stressor on these measures. Rats exposed to a series of uncontrollable but not controllable footshocks develop a transient behavioral deficit upon later shock escape testing. It was of interest to examine the effects of stressor control on changes in DA activity in the terminal regions of the mesocorticolimbic DA system. Male rats were exposed to a series of mild escapable footshocks (ESC), identical inescapable footshocks (INS) or no footshock (CTL) in a yoked triadic design. Within 1 hr of footshock rats were decapitated, and bilateral samples of the PFC, NA and striatum were removed and assayed for DA, DOPAC and HVA. INS induced a bilateral depletion of DA, a large right > left difference in DA utilization (DOPAC/DA) in the PFC and a depletion of DA on the left side of the NA. ESC induced a bilateral activation of DA utilization in the PFC and a left > right difference in DA utilization in the NA. HVA/DA was bilaterally elevated above controls in NA for both the INS and ESC groups. Additional studies have examined these measures at 24hr following footshock. The results indicate that differences in susceptibility to behavioral deficits caused by lack of stressor control may depend upon lateralized brain function. (Support ES04032 J.N.C.)

RECEPTOR MODULATION: UP AND DOWN REGULATION III

519.1

VASOPRESSIN (AVP) RECEPTOR REGULATION FOLLOWING THE SENSITIZATION OF THE RAT BRAIN WITH REPEATED AVP ADMINISTRATION. P.Poulin and Q.J. Pittman. Neuroscience Research Group, University of Calgary, Calgary, Alberta Canada T2N 4N1.

Vasopressin (AVP) administered into the ventral septal area (VSA) of rats produces severe motor disturbances which result from an interaction of AVP with V_1 type of AVP receptors and involve a sensitization process whereby a first administration (sensitization) of AVP causes minor motor disturbances, while a second dose, two days later causes severe motor disturbances. Since repeated AVP injections result in the unusual "sensitization" rather than the more usual "desensitization" phenomenon, we have further investigated it. In a behavioral study, when animals were challenged twice with AVP at different time intervals, we found that the sensitization of the rat brain to AVP induced motor disturbances is time dependent being maximal, 24 hrs following a first injection and lasting for 4 days. If animals were repeatedly challenged with AVP on 6 consecutive days, no tolerance was observed. In binding studies with 3H-AVP as the ligand, Scatchard analysis revealed no differences in Kd or Bmax in VSA membranes from sensitized or control animals (Kd 1.2 vs 0.9 nM and Bmax 28 vs 21 fmol/mg protein, respectively). Thus the mechanism by which such sensitization process takes place is time dependent and does not appear to be via an alteration of AVP receptors affinity or numbers.

519.2

CHRONIC THEOPHYLLINE EFFECTS ON ADENOSINE A1 RECEPTOR BINDING AND IN-VITRO ELECTROPHYSIOLOGY IN THE HIPPOCAMPUS. C.R. LUPICA, M.F. JARVIS* and R.F. BERMAN. Wayne State University, Detroit, MI; *Ciba-Geigy Corp., Summit, N.J.

Chronic treatment with methylxanthines such as caffeine or theophylline (THEO) results in the up-regulation of adenosine A1 binding sites in a variety of neural structures. The present investigation examined the effects of chronic THEO treatment (7d @ 75mg/kg followed by 7d @ 100mg/kg, ip) on evoked responses in hippocampal slices, and on 3H-cyclohexyl-adenosine (CHA) binding in homogenates of contralateral hippocampi from the same subjects. Sixty-four male rats received either THEO or volume-matched saline for 14d. 48hr following the last injection 400uM hippocampal slices were prepared. Field potentials were recorded from stratum pyramidale of area CA1 and the degree of inhibition of this response in adenosine compared across chronic treatment groups. Receptor binding assays using 3H-CHA (0.1-30nM) revealed a significant 20% increase in 3H-CHA binding ($p<.05$) without a change in Kd. THEO treatment also resulted in an increased sensitivity of the field response to 11.2 and 5.0, but not 25uM adenosine ($p<.01$ and .05). These results suggest that chronic adenosine receptor antagonism increases hippocampal A1 binding, and supports the idea that this change in receptor number mediates an increased sensitivity to exogenously applied adenosine.

519.3

PROLONGED ADMINISTRATION OF SOLUFLAZINE DECREASES SPECIFIC BINDING OF ADENOSINE A2 RECEPTORS IN THE RAT STRIATUM. S.D.O'Connor, M.Hawkins, and M. Radulovacki. Dept. of Pharmacology, Univ. IL. Coll. Med., Chicago, Il. 60612.

Recently Ashton et al. (Epilepsy Res. 2:65, 1988) have shown that soluflazine, a water soluble nucleoside transport inhibitor, may act by increasing extracellular adenosine (ADO) levels in the CNS. We have investigated the effect of prolonged treatment (14 days) with soluflazine (20nM/hr, i.c.v.; Alzet miniosmotic pumps) on locomotor activity and ADO receptors in male Sprague-Dawley rats. ADO A1 receptor binding was determined from Scatchard analysis of [3H]R-PIA binding, while ADO A2 receptor binding was estimated by the method of Yeung and Green (Nauyn-Schmiedberg's Arch. Pharmacol., 325:218, 1984). Soluflazine significantly decreased locomotor activity by 38% ($p=0.0397$) during the final 24 hours of drug administration. At the same time, radioligand binding to ADO A2 receptors was significantly decreased by 41% ($p=0.0344$), while no decrease in ADO A1 binding was noted in cortex, hippocampus, cerebellum, or striatum. Previously, we have reported desensitization of ADO A2 receptors in the striatum following prolonged treatment with ADO analogs (Porter et al., JPET, 244: 218, 1988). Our present results further strenghten the hypothesis that soluflazine causes its effects through increases in endogenous ADO.

519.4

CHANGES IN ADENOSINE A2 RECEPTORS IN THE RAT STRIATUM FOLLOWING PROLONGED TREATMENT WITH TRIAZOLAM. M.Hawkins, P.Hajduk *, S.O'Connor, M.Radulovacki and K.E.Starz. Dpts. of Pharmacology, Univ. Ill. Coll. Med., Chicago, Ill. 60612 and Clinical Res., Upjohn Co. Kalamazoo.

We have previously reported that prolonged treatment with diazepam, a benzodiazepine (BZ) with long plasma half-life, attenuates radioligand binding to adenosine (ADO) A2 receptors (Hawkins et al., Neuropharm., 27:1131, 1988). Now we have examined the effects of prolonged treatment (10-20 days) with triazolam (0.5, 1, 2mg/kg/day; s.c. pellets), a BZ with short plasma half-life, on ADO receptors. ADO A1 receptor binding was determined by Scatchard analysis of [3H]R-PIA binding, while A2 receptor binding was estimated by the method of Yeung and Green (Nauyn-Schmiedeberg's Arch.Pharmacol., 325:218, 1984). After 10 days, none of the doses of triazolam altered the number of A1 receptors in all brain areas studied. However, A2 receptor binding was significantly attenuated by 31% ($p=0.003$) in striatal membranes from 2mg triazolam-treated rats. In contrast, the dose of 0.5mg significantly increased radioligand binding to the A2 receptor by 15% ($p=0.001$). Plasma concentrations for the doses of 0.5 and 2mg/kg/day were 0.38 $\pm$ 0.07 and 2.33 $\pm$ 0.77 ng/ml, respectively. After 20 days of treatment none of the doses of triazolam altered radioligand binding to either A1 or A2 receptors. These results suggest that ADO A2 receptors may play a role in the CNS actions of BZ. Supported by a Grant from the Upjohn Co.

519.5

EFFECT OF CORTICOSTERONE ON VIP RECEPTORS IN THE RAT CENTRAL NERVOUS SYSTEM AND PITUITARY. A.Sarrieau*, M.Dussaillant* and W.H.Rostene (SPON: L.Quintin).INSERM U55,184 rue du Faub.St-Antoine 75012 Paris France.

Previous reports showed that the limbic system and the hypothalamo-pituitary axis contain VIP and glucocorticoïd receptors. In these structures, corticosteroids increase or decrease the endogenous level of VIP in the pituitary and the hippocampus respectively. However the effect of glucocorticoïds on VIP receptors has never been studied.

Regulation of VIP binding sites by corticosterone (CORT) was investigated in the hypothalamus, the pituitary and the hippocampus of 1) control, 2) adrenalectomized (ADX), 3) ADX and CORT-implanted, 4) non ADX and CORT-implanted rats for one week.

125I-VIP specific binding was determined from 20µm frontal sections incubated in vitro and processed for autoradiography.

Our results indicate a hypercorticism-induced decrease of VIP receptor density in the subiculum and CA3 of the Ammon's horn, a hypocorticism-induced increase in the supraoptic, periventricular and arcuate nuclei, CA2, CA4 subfields of the hippocampus and in the adenohypophysis; no variation is detected in the suprachiasmatic nucleus and the dentate gyrus.

Our data demonstrate a possible influence of peripheral glucocorticoid hormones on central and pituitary VIP receptors.

519.6

OXYTOCIN PEPTIDE FRAGMENTS AS NEUROMODULATORS-REVERSAL OF UPREGULATION OF PRE- & POST-SYNAPTIC D2 DOPAMINE(DA) RECEPTORS. J.Z. Fields[1,2], R.F. Ritzmann[3] & J.H. Gordon[1,2]. [1]Dept Pharmacol, Loyola Med Sch, Maywood IL; [2]Res Svce, VA Hosp, Hines IL; [3]Brentwood VA Hosp, Los Angeles, CA.

Neuromodulators represent a class of drugs which might alter DA neurotransmission without the acute or chronic side effects associated with direct acting DA receptor (DAr) agonists/antagonists. Pro-leu-gly-NH_2 (MIF-1) and cyclo(leu-gly) (CLG) are oxytocin fragments and prototypes of this class. These peptides inhibit increases in the density of post-synaptic D2 DAr (and associated behaviors) induced by chronic neuroleptics or ovariectomy (Ovx). To evaluate whether CLG can also down-regulate pre-synaptic D2 DAr & to investigate possible molecular mechanisms, we determined the effect of CLG on synthesis-inhibiting DA auto-receptors. Ovx and sham rats were treated with CLG (8 mg/kg/day, SC, X 4 days). On day 5 all rats were given gamma-butyrolactone (GBL; 750 mg/kg, IP); 5 min later APO (1.5 mg/kg, IP) or saline; 30 min later sacrificed. APO induced inhibition (I) of GBL induced increases in DA levels was greater in Ovx/Veh rats (51% I) than Sham/Veh rats (41% I) or Sham/CLG rats (36% I). In Ovx/CLG rats APO had only a 17% I - CLG reversed the Ovx induced DA auto-receptor up-regulation. We hypothesize that CLG somehow increases cleft DA levels & down-regulates all DAr which reverses the behavioral hypersensitivity. (Supported in part by grants from the VA, NIH(NS-26449), Tourette's Syndrome Assn and Scottish Rite Schizophrenia Res Pgm, NMJ)

519.7

TRANSIENT BEHAVIORAL DEFICITS FOLLOWING NEUROTOXIC DOSES OF AMPHETAMINE. L.J. Wichlinski, J.H. Gordon, R.L. Siegel*, P. Charkatz*, and J.Z. Fields. Dept. of Pharmacology, Loyola Univ. Med. School, Maywood, IL; Research Service, Hines VA Hosp., Hines, IL., 60141.

Acute injection of amphetamine (AMPH; 9.2 mg/kg, i.p.) in combination with iprindole (IPRIN; 10.0 mg/kg, i.p.) produces a long-lasting depletion of brain dopamine (DA). We used the same doses and evaluated three treatment regimens, 1, 2, or 3 days of AMPH + IPRIN, for their effects on DA-mediated behaviors. Male F344 rats were tested for spontaneous locomotor activity 1 wk or 3 wks after the conclusion of drug treatment, and for apomorphine (APO) induced stereotypy (repetitive, non-exploratory sniffing) at 10 days. At 1 wk, animals injected 1x or 3x with AMPH + IPRIN showed suppressed horizontal and vertical activity during the first 5 min of testing. By 3 wks, the 1x group had recovered to control activity levels. APO-induced stereotypy was increased in rats injected 1x or 3x with AMPH. The decrease in activity may be related to the AMPH-induced depletion of DA. The increase in APO-induced stereotypy suggests that the recovery of activity may be associated with an up-regulation of post-synaptic DA receptors. (Supported in part by the VA; NIH #NS-26449; Tourette's Syndrome Assoc.; and the Scottish Rite Schizophrenia Research Program, N.M.J., U.S.A.)

519.8

COMPARISON OF LONG-TERM EFFECTS OF LITHIUM AND CARBAMAZEPINE ON PHOSPHOINOSITIDE TURNOVER IN CULTURED CEREBELLAR GRANULE CELLS. X.-M. Gao and D.-M. Chuang (Spon: D. Kirch). Lab. of Preclinical Pharmacol. NIMH, St. Elizabeths Hosp., Washington, DC 20032.

The mechanisms of action of lithium (Li^+) and carbamazepine (CBZ) for the treatment of manic depression are still unclear. We have studied effects of Li^+ and CBZ on basal and muscarinic cholinergic receptor-mediated phosphoinositide (PI) turnover in cerebellar granule cells. 3-Day exposure of cultured cells to Li^+ affected biphasically basal and carbachol-stimulated ^{3}H-inositol monophosphate accumulation. At low concentrations (< 10 mM) of Li^+, basal and carbachol-induced PI turnover was enhanced; at higher concentrations (10-50 mM), these activities were markedly attenuated. The inhibition of carbachol response observed with 20 mM Li^+ was associated with a major reduction of the maximal stimulation. The enhancement induced by 2 mM Li^+ was time-dependent with activities progressively increased between day 2 and 7. 3-Day exposure of granule cells to low concentrations (< 50 μM) of CBZ had no substantial effects on basal and receptor-mediated PI turnover; however, higher concentrations (50-100 μM) of CBZ induced a marked attenuation of carbachol-induced PI breakdown. This inhibition of carbachol response was reversed by longer-term (7-day) exposure to CBZ. Thus, Li^+ amd CBZ appear to have complex but distinct effects on PI turnover in cerebellar granule cells.

519.9

ETOPERIDONE, A NOVEL ANTIDEPRESSANT, MAY DOWN-REGULATE HUMAN PLATELET IMIPRAMINE RECEPTORS. JR Magliozzi, DW Gietzen, S Wimberg* and DJ Donohoe*. Dept. Psychiatry, Univ. Cal. (Davis) Sch. Med. and McNeil Pharmaceutical Inc., Spring House PA.

Classical antidepressants are thought to bind to imipramine receptors (IR's), although the newer antidepressant agents have not been well characterized in this regard. We investigated changes in Bmax of the IR in 17 depressed patients randomized to treatment with etoperidone HCl (ETO) or placebo (PLA). Platelet membranes were harvested prior to and at 6 and 14 weeks of treatment. The IR was defined by binding of ^{3}H-imipramine in the presence of 10 micromolar desipramine. IR Bmax declined in both groups (for ETO: 642.3, 470.4 and 408.1 fmoles/mg platelet membrane protein; for PLA: 657.4, 426.0 and 521.4, at baseline, 6 and 14 weeks). Comparison of IR Bmax with scores of the Hamilton Depression Scale (HAMD) using a multivariate repeated measures analysis indicated that at a given level of anti-depressant response, IR Bmax in the ETO group was significantly lower than in the PLA group (Lambda=0.52, $F_{(2,10)}$=4.54, p=0.04). These results suggest that ETO may downregulate platelet IR's in parallel with its antidepressant efficacy.

519.10

EFFECT OF CHRONIC ADMINISTRATION OF ANTIDEPRESSANT DRUGS ON SEROTONIN TYPE 2-MEDIATED BEHAVIOR IN THE RAT FOLLOWING NORADRENERGIC OR SEROTONERGIC DENERVATION. A.S. Eison, F.D. Yocca, and G. Gianutsos. Section of Pharmacology and Toxicology, School of Pharmacy, Univ. of Connecticut, Storrs, CT 06268 and CNS Research, Bristol-Myers Co., Wallingford, CT 06492-7660.

Chronic (14 day) administration of several pharmacologically-distinct antidepressant drugs resulted in marked reductions in the quipazine-induced head shake response which were accompanied by significant reductions in the density of cortical beta-adrenergic and 5-HT_2 binding sites. Noradrenergic (DSP4-induced) and serotonergic (5,7-DHT-induced) lesions either attenuated or blocked antidepressant-induced reductions in 5-HT_2-mediated behavior. DSP4- and 5,7-DHT lesions did not alter the down-regulation of 5-HT_2 binding sites by imipramine, desipramine, phenelzine or iprindole. To a large extent, the antagonism of antidepressant-induced reductions in 5-HT_2-mediated behavior was coincident with the blockade of down-regulation of beta-adrenergic binding sites by both noradrenergic and serotonergic denervation. The functional inter-relationship of 5-HT_2 and beta-adrenergic receptors suggested by the present findings may provide insight into a common mechanism underlying the action of pharmacologically-distinct antidepressant drugs.

519.11

DIFFERENTIAL EFFECTS OF VARIOUS ANTIDEPRESSANT TREATMENTS ON M-CPP-INDUCED DECREASES IN FOOD INTAKE IN FAWN-HOODED RATS. C.S. Aulakh,* J.L. HILL,* and D.L. Murphy* (SPON: J.M. Tolliver). LCS, National Institute of Mental Health, Bethesda, MD 20892.

We recently demonstrated that the Fawn-Hooded (FH) rat strain is functionally subsensitive to m-chlorophenylpiperazine (m-CPP, a 5-HT_1 agonist)-induced decreases in food intake (Psychopharmacology 94: 588-592, 1988) and locomotor activity (Pharmacol. Biochem. Behav. 31: 567-571, 1989), and increases in plasma prolactin (Neuroendocrinology 48: 401-406, 1988) relative to Wistar and Sprague-Dawley rat strains. In the present study, we used m-CPP as a challenge agent to explore functional adaptational changes in serotonergic neurotransmission involved in regulation of food intake following long-term treatment with imipramine (a tricyclic antidepressant), clorgyline (a selective MAO type A inhibiting antidepressant), and lithium in male FH rats.

M-CPP-induced decreases in food intake were significantly attenuated by long-term (21-25 day) but not short-term (3-5 day) treatment with clorgyline. In contrast, food intake responses to m-CPP were potentiated by long-term treatment with both imipramine and lithium. These findings demonstrate that various antidepressant treatments in the FH rat strain produced differential effects, which were also different, in part, from those observed in our previous studies in the Wistar rat strain (Eur. J. Pharmacol. 94: 175-179, 1983; Br. J. Pharmacol. 91: 747-752, 1987; Psychopharmacology in press).

519.12

ANTIDEPRESSANT-INDUCED DOWN REGULATION OF CENTRAL β_1 ADRENOCEPTORS: REGIONALLY SELECTIVE EFFECTS. **P. Areso*, C. Gambarana, S. Tejani-Butt, M. Hauptmann* & A. Frazer. Vet. Adm. Med. Ctr. & Univ. of Pennsylvania, Phila., PA 19104.**

In the present study, effects of different antidepressants on subtypes of β-adrenoceptors (BARs) were examined in many areas of brain using quantitative autoradiography (*Proc. Natl. Acad. Sci.* (USA), 81:1585, 1984). Chronic administration of antidepressants that acutely have direct effects on the uptake of NE (desipramine & protriptyline), its enzymatic metabolism (phenelzine & tranylcypromine) or blockade of α_2-adrenoceptors (mianserin) caused a reduction in total binding of ^{125}I-iodopindolol (^{125}I-IPIN) in discrete areas of brain. All of the antidepressants studied decreased the binding of ^{125}I-IPIN to the β_1 subtype. The most consistent and robust effect in reducing binding to the β_1 subtype was seen for all of these drugs in certain nuclei of the amygdala. Antidepressants that do not have potent effects on noradrenergic neurons (sertraline & trazodone) caused no significant effect on ^{125}I-IPIN binding to either subtype of BAR in any area of brain examined. Only phenelzine decreased binding of ^{125}I-IPIN to the β_2 subtype. Thus, only antidepressants with potent acute affects on noradrenergic function caused a decrease in β_1 receptors and such drugs produced this effect most consistently in the amygdala. **(Supported by the Vet. Adm., USPHS Grant MH29094 & an MEC Fellowship from Spain.)**

519.13

ENHANCED YAWNING RESPONSE TO THE DOPAMINE D-2 AGONIST, LY171555, IN ADULT RATS THAT WERE TREATED IN DEVELOPMENT WITH LY171555. A. Hamdi and R.M. Kostrzewa (SPON: M.D. Miyamoto). Quillen-Dishner College of Medicine, East Tennessee State Univ., Johnson City, TN 37614.

Because dopamine D1 and D2 receptor antagonists, alter development of striatal D1 and D2 receptors, respectively, we determined how the D1 and D2 agonists (SKF38393, LY171555) and antagonists (SCH23390, spiroperidol), respectively, would alter the yawning response to LY171555 in rats that were treated in development with the above agents. Rats were treated once a day i.p. for 32 consecutive days from birth with SCH23390 (0.3 mg/kg/d), spiroperidol (0.3 mg/kg/d), SKF (3.0 kmg/kg/d), or LY 171555 (3.0 mg/kg/d) with or without 6-hydroxydopamine (6-OHDA; 133 ug ivtr, at age 3d), and were challenged with LY171555 (50 ug/kg) at 5 to 6 weeks of age. The incidence of yawning was increased in the LY171555 group, but decreased in the SCH23390 group. Treatment in development with spiroperidol or SKF38393 did not alter the yawning response. 6-OHDA eliminated the yawning response in the SKF38393, LY171555, and control groups. The SCH23390 groups, with or without 6-OHDA, gave the same response, which was less than the control group. These findings indicate that treatment only with a D2 agonist in development will enhance the D2 mediated yawning response in rats that are studied as adults. (Supported by BMRG 2 SO7RR05959)

519.14

ENHANCED STEREOTYPIC RESPONSES TO DOPAMINE AGONISTS IN ADULT RATS THAT WERE TREATED IN DEVELOPMENT WITH SKF38393. R.M. Kostrzewa and A. Hamdi. East Tennessee State University, Quillen-Dishner College of Medicine, Johnson City, TN 37614.

Because the dopamine D1 receptor antagonist, SCH23390, alters development of striatal D1 receptors, we studied how developmental treatment with a D1 agonist would affect responses in adulthood. Rats were treated once a day for 32 days from birth with SKF38393 (3.0 mg/kg/d, i.p.), and were challenged with the D1 agonist at 6 to 12 weeks of age. SKF38393 increased locomotor activity, as well as the incidence of digging, gnawing, and rearing. Rats in the SKF 38393 group that were given an additional neonatal treatment with 6-hydroxydopamine (133 ug, ivtr at 3d) had enhanced licking, grooming, digging, taffy pulling, jumping and locomotor stereotypic responses. These findings indicate that D1 agonist treatment of developing rats will modify subsequent responses to a challenge dose of the agonist. The new animal models provide additional means of studying the dopamine receptor system. (Supported by BMRG 2 SO7RR05959)

519.15

NEUROLEPTIC ACTION ON RAT BRAIN SIGMA RECEPTORS. Tyrone Lee and M. Noella Picarelli*. Psychopharmacology Unit, Clarke Institute of Psychiatry, Toronto, Canada.

HP370 and BMY14802 are new antipsychotics which cause less catalepsy in rats, suggesting non-dopaminergic action in the CNS. The postulated role of σ receptors in mediating some neuroleptic effects prompted us to investigate if these agents alter rat brain σ receptors in vivo, in comparison with the classical haloperidol and atypical loxapine.

Male Wistar rats were given single injection i.p. of haloperidol, loxapine, HP370 or BMY14802 at doses of 5, 5, 20 and 10 mg/kg, respectively. They were sacrificed 24 hours later and their brain σ receptors were measured using ^{3}H-N-allylnormetazocine. Receptor density and affinity changes are reported as follows:

DRUGS	n	B_{max}	%	K_D	%
Control	(8)	55 ± 13#	100	36 ± 9	100
Haloperidol	(8)	12 ± 2	22*	21 ± 3	57
Loxapine	(8)	49 ± 13	89	27 ± 6	75
HP370	(4)	61 ± 11	111	27 ± 6	75
BMY14802	(4)	55 ± 16	100	28 ± 10	79

#Average ± S.E.M.; * P<0.01, Student's t-test.

The present results indicate HP370 and BMY14802, like loxapine, did not seem to alter the σ receptors acutely whereas haloperidol significantly reduced the receptor number compared to controls. (Supported by the Clarke Inst.)

519.16

DIFFERENTIAL REGULATION OF ALPHA-1 ADRENERGIC RECEPTOR SUBTYPES BY RESERPINE. L.J. Grimm[1], J.A. Blendy[2], L. West-Johnsrud[2], D.C. Perry[1] and K.J. Kellar[2]. Depts. of Pharmacology, [1]George Washington Univ., Washington, DC 20037 & [2]Georgetown Univ., Washington, DC 20007

Chronic reserpinized rats show increases in cortical homogenate binding of 3H-prazosin (3H-PZ) to α-1 adrenergic receptors but no change in 3H-WB4101 (3H-WB) binding (Eur J. Pharm 139: 259 1987). To further characterize the effects of chronic reserpine treatment (1 mg/kg day 1-2, 0.5 mg/kg day 3-15) on α-1 receptors in rat brain, we used quantitative autoradiography with 3H-PZ (0.9nM), which labels both α-1a and α-1b sites. Reserpine produced large increases in 3H-PZ binding in thalamic nuclei (40-60%) and cortical regions (20-30%), and smaller increases in some areas of the amygdala and hippocampus (10-15%). Binding in other brain regions was not significantly affected. Binding with 3H-WB (0.39nM), which labels primarily α-1a sites (with non-specific binding determined by 60 nM PZ), was relatively homogeneous throughout the brain (unlike 3H-PZ binding which varied in density over a 5-fold range). No significant changes were observed in 3H-WB binding to rat brain following reserpine (with the exception of increased binding (21%) in insular cortex), suggesting that chronic reserpine increases α-1b receptors but not α-1a. Supported by MH41819 (KJK) and NSF BN8516196 (DCP).

519.17

ELECTROCONVULSIVE SHOCK INCREASES ONE SUBTYPE OF ALPHA-1 ADRENERGIC RECEPTORS IN SELECTIVE AREAS OF RAT BRAIN.

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3H-Prazosin (PZ) labels two subtypes of α-1 receptors with equal affinity. These subtypes are discriminated on the basis of their affinities for the antagonist WB4101. The subtype with high affinity for WB4101 has been termed α-1a, while that with low affinity has been termed α-1b. Previous results have shown that repeated administration of electroconvulsive shock (ECS) increases α-1 adrenergic receptors as labeled by 3H-PZ. In the present study we have examined the question of whether one or both subtypes are affected by ECS by including WB4101 in our 3H-PZ binding experiments at concentrations which mask the α-1a subtype receptor. Our results demonstrate that 3H-PZ binding in homogenate preparations of frontal cortex is increased by 24% in ECS animals compared to control (N=6). The addition of 2 or 10nM WB4101 to the binding assay in the same tissues results in 49% or 60% increases, respectively, in the treated animals. Quantitative autoradiography findings showed that 3H-PZ binding in the cortex was increased by 20-30% in ECS animals, however in the presence of 10 nM WB4101 the increases were in the range of 30-75%. In addition, areas of the amygdala showed an increase in 3H-PZ binding in ECS animals, but unlike the cortex this increase does not appear to be affected by the addition of WB4101. These findings indicate that ECS selectively increases the α-1b adrenoceptor subtype in most cortical areas of rat brain.

519.18

ALTERATIONS IN THE BINDING CHARACTERISTICS OF GLUCOCORTICOIDS IN OBESE ZUCKER RATS. B.D. White* and R.J. Martin. Dept. of Foods and Nutrition, University of Georgia, Athens, GA 30602.

Obese Zucker rats have been shown to lack a circadian rhythm of plasma corticosterone. Elevated morning concentrations of plasma corticosterone in obese rats result in relatively high corticosterone concentration throughout the 24-hour day. The purpose of this study was to characterize glucocorticoid receptors in brain regions known to be involved in negative feedback to determine if receptor alterations may be involved in the lack of a corticosterone rhythm. Eight obese and ten lean male Zucker rats (3 mo. old) were adrenalectomized and sacrificed within 24 hours. The brains were removed and the anterior pituitary, hypothalamus, and hippocampus isolated. Additionally, liver tissue was taken. Tissues were homogenized in a Tris buffer and the cytosolic fractions were collected by ultracentrifugation. Radioreceptor assays were performed using tritiated dexamethasone (0.1-30nM). Nonspecific binding was determined by the addition of 1000-fold excess of cold dexamethasone. The Kd of binding was higher in the anterior pituitary of obese rats than in lean rats. No other phenotypic difference in binding was observed in the brain regions. Liver tissue from obese rats showed both a higher Kd and a lower maximal binding as compared to lean rats. Lower maximal binding in the liver of obese rats may reflect down-regulation of glucocorticoid receptors by higher daily concentrations of plasma corticosterone. A higher Kd in the anterior pituitary suggests a lower sensitivity to glucocorticoids. Thus, it is possible that obese Zucker rats have less feedback inhibition at the level of the anterior pituitary.

519.19

ALTERATIONS IN ^{3}H SCH 23390 BINDING IN RAT BRAIN FOLLOWING PERINATAL EXPOSURE TO COCAINE. D.H. Segal*, L.A. Freed, H.E. Hughes, T.H. Milhorat and D.L. Dow-Edwards. (SPON: E. Elowitz). Lab of Cerebral Metabolism, Dept. of Neurosurgery, SUNY-Health Science Center, Brooklyn, NY 11203.

Cocaine abuse is currently regarded as one of the major threats to our societal infrastructure. We have demonstrated long-term changes in functional activity of dopaminergic brain regions following perinatal cocaine exposure in the rat. The present study examines the effects of perinatal cocaine exposure on the development of dopaminergic receptors. Rats were administered cocaine, 50 mg/kg sc, each day between day 1 and 10 or between day 11 and 20. On postnatal day 60-70, central dopamine receptors were identified with ^{3}H SCH 23390, a ligand with high selectivity for the D_1 receptor using quantitative autoradiography.

Treated rats showed an increase in ligand binding in a number of cerebral structures including those of the limbic and motor systems compared to vehicle injected controls. Many of these structures were previously shown to have increased metabolic activity in adulthood following perinatal exposure to cocaine. These data suggest that cocaine consumption during pregnancy may be responsible for long term functional alterations in the dopaminergic systems in brain.

Supported by NIDA Grant # DA04118.

519.20

CORRECTION OF AUTORADIOGRAPHIC TRITIUM QUENCHING

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Accurate quantification of autoradiograms generated by tritiated probes is hampered by the phenomenon of differential tissue quenching. Gray and white matter containing equivalent amounts of tritium will generate different autoradiographic signals because white matter absorbs more of the low energy beta-emissions than gray matter. These experiments investigate an autoradiographic method for the detection and correction of tissue quenching.

Our method involves the autoradiography of blocks of tritium-impregnated plastic overlayed with thin unlabeled brain sections. In this study, tissue overlays of coronal sections obtained from hippocampal and cerebellar levels were prepared. The autoradiographic image cast through the cortical regions of a ten micron cerebellar section were three times more efficient at representing the tritium concentration of the plastic background than the image over deep white matter.

The effect of tissue thickness on autoradiographic efficiency was examined. Gray matter efficiencies ranged from 43% to 17% for sections between 8 and 14 microns thick. White matter efficiencies were 18% to 6% for the same range of section thickness. In addition, we observed that the ratio of white matter overlay efficiency to gray matter efficiency varied with tissue thickness.

This latter result suggests that overlay efficiencies and the efficiencies of labeled sections may not be directly proportional and that a complex mathematical analysis will be required to convert overlay results to a measure of regional tissue quenching. Despite this difficulty, overlay images still can provide a means of digital quench correction for autoradiographic data obtained from adjacent sections.

519.21

Chronic verapamil treatment affects imipramine binding to human platelets. M. Rehavi*, A Cohen*, M. Eldar*, R. Carmi* and A. Weizman*. Dept. of Physiology and Pharmacology, Sackler Faculty of Medicine, Tel-Aviv University, Tel-Aviv 69978, Israel. (SPON: N. Dascal)

Several case reports have suggested that calcium channel blockers, mainly verapamil, are effective in the treatment of affective disorders. Verapamil was found to be a competitive inhibitor of both [^{3}H]imipramine binding and [^{3}H]serotonin uptake to rat brain and human platelets (Ki=0.82-4.29μM). Two other calcium channel blockers, nitrendipine and diltiazem were inactive at the serotonin transporter site. Tricyclic antidepressants inhibited (-)[^{3}H]desmethoxyverapamil binding (Ki=0.21-1.07μM) but did not affect [^{3}H]nitrendipine binding to rat cerebral cortex membranes. Increased density of [^{3}H]imipramine binding sites (24%, $p<0.035$) was detected in platelets of cardiac patients maintained on chronic (at least two months verapamil treatment (80-360 mg/day) when compared to normal healthy age and sex-matched volunteers. The chronic treatment did not affect the affinity of imipramine to its binding site. Modulatory effect of in vivo chronic verapamil treatment on the serotonin transporter is suggested.

519.22

DOPAMINE INCREASES THE OPENING FREQUENCY OF KAINATE-SENSITIVE ION CHANNELS IN WHITE PERCH HORIZONTAL CELLS. Karl F. Schmidt*, Andrew G. Knapp, and John E. Dowling, The Biological Laboratories, Harvard Univ., Cambridge, MA.

Dopamine enhances responses of cultured white perch cone horizontal cells to kainate and L-glutamate (Knapp and Dowling, Nature 325, 437). We have used whole-cell current noise analysis and single-channel recordings to determine those properties of the excitatory amino acid-gated channels (i.e. conductance, number, kinetics) that change following exposure of the neurons to dopamine. When the levels of kainate or L-glutamate were raised or lowered around voltage-clamped horizontal cells by slow superfusion, the mean agonist-induced current was parabolically related to the variance of the current. Addition of dopamine (200 μM) to the superfusate increased the mean current but changed neither the mean vs. variance relationship nor the power spectrum of the agonist-induced currents, suggesting an increase in channel open probability but not in mean channel open time. In single-channel recordings from cell-attached patches with kainate or L-glutamate in the pipette, dopamine approximately doubled the frequency of 5-10 pS channel openings, without altering the size of the unitary events, and only slightly increasing the mean channel open time. These results suggest that dopaminergic enhancement of responses to excitatory amino acids in horizontal cells is mediated predominantly by an increase in the frequency of channel opening.

SECOND MESSENGERS: ADENYLATE CYCLASE

520.1

INTERACTIONS BETWEEN ADENYLATE CYCLASE AND PHOSPHATIDYLINOSITOL (PI) SIGNALLING SYSTEMS IN NCB-20 CELLS. C.L. Boyajian and D.M.F. Cooper*. Department of Pharmacology, University of Colorado Health Sciences Center, Denver, CO 80262.

Individual signal transduction systems within cells appear to interact at a number of levels. We have been studying the interplay between Ca^{2+}-mobilizing and cAMP-generating systems. In most brain regions, Ca^{2+} concentrations corresponding to those achieved upon membrane depolarization or activation of the PI system, stimulate adenylate cyclase activity via calmodulin. Recently, however, we have found that these concentrations elicit a profound (~45%) inhibition of the plasma membrane adenylate cyclase activity of NCB-20 cells (a neuroblastoma x hamster striatum hybrid cell line). This effect is entirely independent of calmodulin, yet highly cooperative (n_H=1.67) for Ca^{2+} ions. It requires a stimulated activity state of cyclase, and is not sensitive to the actions of pertussis toxin. In intact NCB-20 cells, bradykinin, which stimulates PI hydrolysis and Ca^{2+}-mobilization, causes a significant inhibition of cAMP production, which is not due to any stimulation of cAMP phosphodiesterase activity. These data indicate that considerable crosstalk occurs between Ca^{2+}-mobilizing and cAMP-generating systems, which may vary across cell types in accomodating specific physiological demands.

520.2

α_1 RECEPTOR AUGMENTATION OF β-RECEPTOR STIMULATED CYCLIC AMP FORMATION IS MODIFIED BY OVARIAN STEROIDS. N. Petitti and A.M. Etgen. Depts. of Psychiatry and Neuroscience, Albert Einstein College of Medicine, Bronx, NY, 10461.

Norepinephrine (NE)-stimulated cyclic AMP (cAMP) accumulation in hypothalamic and preoptic area slices is modified by estradiol (E_2) and progesterone (P). NE elicits greater cAMP accumulation in slices from E_2-treated female rats than in slices from E_2+P-primed animals. Present studies examined the possibility that the α_1 receptor augmentation of β receptor-stimulated cAMP synthesis seen in E_2-treated rats is eliminated by P. In slices from E_2-primed rats, cAMP levels were elevated by the β agonist isoproterenol (ISO) in a concentration-dependent manner. Addition of the α_1 agonist phenylephrine (PHE) shifted the ISO concentration-response curve upwards. In slices from E_2+P-treated rats, the ISO concentration-response curve was the same with or without PHE. Further experiments assessed whether the loss of α_1 augmentation of cAMP accumulation in E_2+P-treated rats was due to the loss of α_1 receptor activation of protein kinase C. Phorbol-12,13-dibutyrate, a direct activator of protein kinase C, restored NE-stimulated cAMP levels in E_2+P-exposed slices to those seen in slices treated only with E_2. The results indicate: (1) that α_1 receptor augmentation of β-stimulated cAMP formation in E_2-primed rats is probably mediated by the action of protein kinase C, and (2) that P treatment of E_2-primed rats causes a loss of this α_1 receptor-mediated synergy.

520.3

EFFECTS OF CYCLIC 3',5'-ADENOSINE MONOPHOSPHATE ON RAT SPINAL DORSAL HORN NEURONS IN VITRO. R. Cerne*, G. Gerber*, I. Kangrga and M. Randic (SPON: W. G. VanMeter). Dept. of Vet. Physiol. and Pharmacol., Iowa State University, Ames, IA 50011, USA.

Intracellular recordings were made from rat spinal dorsal horn neurons in the in vitro slice preparation to study the actions of cyclic 3',5-adenosine monophosphate (cyclic AMP). Bath application of the membrane permeant analogue of cyclic AMP, 8-Br cyclic AMP produced a slow depolarization of the resting membrane potential, an increase in membrane input resistance and excitability. Activation of the endogenous adenylate cyclase of dorsal horn cells by bath application of forskolin, or inhibition of the phosphodiesterase activity by methylxanthines, resulted also in a slow depolarization accompanied by an increase in "spontaneous" synaptic activity and enhancement of dorsal root-evoked EPSPs. In the presence of TTX, 8-Br cyclic AMP and forskolin enhanced, in a reversible manner, the depolarizing responses of a proportion of dorsal horn neurons to N-methyl-D-aspartate (NMDA) ejected extracellularly by positive pressure from micropipettes. The enhanced responsiveness to NMDA is potentiated in a zero-Mg^{2+} solution and at depolarized membrane potentials when 1mM Mg^{2+} was present. The enhancing effect was reduced by H-8, an inhibitor of cyclic AMP-dependent protein kinase. These results suggest the possibility that activation of the adenylate cyclase and cyclic AMP-dependent protein kinase system may be involved in regulation of excitability and sensitivity of postsynaptic NMDA receptors in the rat spinal dorsal horn neurons. Supported by NIH, NSF and USDA.

520.4

D1 RECEPTOR COMPLEX SUPERSENSITIVITY RESULTS IN A DECREASED INHIBITORY RESPONSE OF ADENYLATE CYCLASE. M.G.De Montis, P.Devoto*, A.Porcella*, P.Saba* and A.Tagliamonte*. Inst. of Pharmacology and Biochem. Pathology, University of Cagliari, Italy.

Rats chronically exposed to SCH 23390, a selective D1 dopamine (DA) receptor blocker, show increased number of striatal D1 binding sites (+32%) and increased adenylate cyclase response to DA stimulation. DA supersensitivity was due to an increased amount of the enzyme, since both DA and Forskolin stimulation produced a marked increase of the Vmax. In these animals the inhibition od DA - stimulated striatal adenylate cyclase activity by opiates (DADLE and Dynorphin,1-13) was the same in control rats and in rats chronically exposed to SCH 23390. On the other hand, the increased Vmax of adenylate cyclase produced by long term D1 receptor blockade was accompanied by a significantly decreased response of basal enzyme activity to the inhibitory effect of both opiates and muscarinic agonists. We conclude that the endogenous inhibitory tonus on adenylate cyclase activity is increased after chronic SCH 23390 treatment.

520.5

TUBULIN STIMULATES ADENYLATE CYCLASE IN C6 GLIOMA MEMBRANE VIA TRANSFER OF GUANINE NUCLEOTIDE FROM TUBULIN TO THE STIMULATORY G-PROTEIN, Gs. Y. Kun, N. Wang & M.M. Rasenick. (Spon: R. Greenberg) Department of Physiology & Biophysics, University of Illinois College of Medicine, Chgo, Illinois, 60680.

It has been shown that in synaptic membranes, brief incubation with tubulin polymerized with GppNHp (tubulin-GppNHp) inhibits adenylate cyclase in a dose-dependent manner. This inhibition persists after washing and appears to be due to the direct guanine nucleotide transfer from tubulin to Gi as verified by azidoanilido-GTP photolabeling (J. Neurochem., 51:300-311, 1988). Curiously, in C6 glioma membranes, tubulin-GppNHp stimulates adenylate cyclase activity under the same experimental conditions. This stimulation is also dose-dependent and saturable. It is noteworthy that there are at least two relevant differences between synaptic and C6 glioma membranes. First, they have different Giα subtypes (Gi1α mRNA has not been detected in C6 glioma cells). Secondly, in C6 glioma membranes, the β-adrenergic receptor is tightly coupled to the stimulatory G-proteins, Gs. This coupling may exert some constraint on the G-proteins in C6 membranes whereas in synaptic membranes, this constraint does not exist. In a series of hybridization experiments, we have demonstrated that [^{125}I]-tubulin binds to Gsα and Gi1α with a significantly higher affinity that to other Gα subunits. This result supports our hypothesis that the lack of Gi1 in C6 membrane may be one of the explanations for the discrepancy between C6 and brain membrane systems. In saponin-treated C6 cells, tubulin-GppNHp stimulates adenylate cyclase in the absence of agonist, in contrast, free GppNHp could not affect adenylate cyclase activity under the same condition. This result implies that tubulin-GppNHp is able to bypass the receptor and access Gs directly. Supported by MH 39595 and NSF:BNS 87-19758.

520.6

TUBULIN-G PROTEIN INTERACTION AND NUCLEOTIDE EXCHANGE: REGULATION OF NEURONAL ADENYLATE CYCLASE. N. Wang, Y. Kun and M.M. Rasenick. Physiology and Biophysics, Univ. of Illinois, College of Medicine, Chicago, IL. 60680

Tubulin, the primary constituent of microtubules, is a GTP-binding protein with structural similarities to other GTP-binding proteins. Whereas, microtubules have been implicated as modulators of the adenylate cyclase system, the mechanism of this regulation has been elusive. Tubulin, polymerized with the hydrolysis-resistant GTP analog, GppNHp, promoted inhibition of synaptic membrane adenylate cyclase which persisted subsequent to membrane washing. When tubulin was polymerized with the hydrolysis-resistant photoaffinity GTP analog, [^{32}P]-AAGTP, and this protein was incubated with synaptic membranes, AAGTP was transferred from tubulin to the inhibitory GTP-binding protein, Gi. Control experiments performed showed that this transfer of nucleotides from tubulin to Gi was via a direct process and it was not due to releasing of nucleotide from tubulin, then rebinding to Gi. Tubulin, regardless of the nucleotide bound to it, had no direct effect on the adenylate cyclase catalytic moiety. Western blotting and dot blotting hybridization with [^{125}I] tubulin as well as immunoprecipitation showed a direct interaction between tubulin and α subunits of G proteins. This interaction is specific and distinct for different subtypes of Gα. These data support the hypothesis that tubulin forms complexes with G proteins and, in doing so, may later alter neuronal adenylate cyclase via the transfer of GTP. Supported by PHS MH 39595 and MH 00699.

520.7

EFFECTS OF PEPTIDES ON ADENYLATE CYCLASE ACTIVITY IN RAT CORTICAL SYNAPTOSOMES. G.S. Dhillon & M.L. Koenig. Dept. of Medical Neurosciences, Walter Reed Army Institute of Research, Washington D.C. 20307-5100.

A number of peptides and physiologically active substances have been reported to affect the neuronal activity and release of neurotransmitters by affecting second messenger systems (cAMP-dependent and/or calcium-dependent mechanisms). In the present study we investigated the effects of a number of peptides with neuromodulatory properties on adenylate cyclase activity in synaptosomes prepared from rat cortex.

Synaptosomes were prepared from freshly dissected rat brain cortex by the method of Booth and Clark (Biochem. J. 176:365-370, 1978). Adenylate cyclase activity was measured in P_3 fractions by the method of Solomon et al (Analytical Biochem.58:541-548, 1974). CRH (1 uM), VIP (1 uM) and NE (50 uM) increased basal adenylate cyclase activity by 80%, 118% and 75% respectively. The effects of CGRP, NPY and PTH (1 uM) were less pronounced, producing increases in adenylate cyclase activity of 34%,16% and 19% respectively. Somatostatin (1 uM) and isoproteranol (50 uM) had no effects on the enzyme activity. Vasopressin (1 uM) alone or in combination with CRH did not affect the adenylate cyclase activity. These results indicate that at least some of these peptides may exert their neuromodulatory effects through cAMP-dependent mechanisms.

520.8

DOPAMINE SIGNAL TRANSDUCTION IN THE AMYGDALOID COMPLEX. J.E. Lachowicz, C.M. Anderson and C.D. Kilts. Dept. of Pharmacology, Duke Univ. Med. Ctr., Durham, N.C. 27710.

Transduction of the dopamine (DA) signal by D_1 receptors in the CNS is thought to involve a cascade of G proteins, adenylate cyclase, cyclic AMP dependent kinase and phosphoproteins (e.g., DARPP-32). However, it is increasingly evident that D_1 receptors in the amygdaloid complex do not initiate this cascade. We have corroborated our findings with cell-free preparations (Kilts et al., Neurosci. Abs. 14:78, 1988) by measuring cAMP efflux from superfused tissue slices. While D_1 receptor activation increases cyclic AMP efflux from neostriatum, D_1 agonists have no such effect on amygdala slices. Conversely, isoproterenol significantly increases cyclic AMP efflux from amygdala slices. Thus, β-noradrenergic but not D_1 receptors in the amygdaloid complex are coupled to the adenylate cyclase second messenger system.

Ongoing investigation of DA signal transduction in the amygdaloid complex focuses on the inhibitory influence of D_2 receptors on cyclic AMP efflux, DA receptor regulation of G protein function and phosphoproteins, and the interaction of D_1 and D_2 receptors on these putative transducer proteins and second and third messengers. (Supported by MH-39967)

520.9

EFFECTS OF DOPAMINE ON POTASSIUM PERMEABILITY IN RAT LACTOTROPHS. A. Valerio* M.Memo , L. Castelletti* E. Nisoli* C. Missale, P.F. Spano. Inst Pharmacol Exp Ther, Sch Med, Univ Brescia, Italy.

It is generally accepted that D-2 dopamine (DA) receptor activation inhibits adenylate cyclase (AC) activity. There is now evidence that inhibition of AC may not be entirely responsible for D-2-mediated effects of DA. The effects of DA on K permeability were investigated in purified lactotrophs with a manual rapid-quench method where the evaluation of 86Rb efflux was taken as an index of K permeability. Both voltage-activated and Ca -activated K fluxes have been identified in mammotrophs. DA and D-2 agonists affected both types of 86Rb efflux in a Ca -free medium containing 5 mM K ,DA significantly increased Rb efflux. DA also increased Rb efflux in a 5 mM K solution containing 1 mM Ca ; this effect was rapid (2-5 sec), was inhibited by 0.5 mM cadmium and appeared to be mediated by AC inhibition.

These data support the view that there are multiple tansduction mechanisms for D-2 receptors involving either inhibition of AC on the increase in K permeability.

520.10

GABA$_B$ RECEPTOR ACTIVATION INHIBITS Ca^{2+}-ACTIVATED K^+-CHANNELS VIA G-PROTEIN MECHANISM IN SPINAL CORD NEURONS. G. Kamatchi* and M. K. Ticku (SPON: D. Ross). Dept. of Pharmacology, Univ. of Texas Hlth. Sci. Ctr., San Antonio, TX 78284-7764.

The effect of GABA$_B$ receptor activation on Ca^{2+}-activated K^+-efflux was studied in primary cultured spinal cord neurons by using ^{86}Rb. The ^{86}Rb-efflux significantly increased after depolarization of the cultured cells with 100 mM KCl. This efflux was blocked partly by quinine sulfate, tetraethyl ammonium and La^{3+}, indicating the involvement of Ca^{2+}-activated K^+-channels. The GABA$_B$ agonists (-)baclofen and GABA inhibited this Ca^{2+}-activated ^{86}Rb-efflux. This inhibition seems to be mediated through GABA$_B$ receptors, since it was blocked by GABA$_B$ antagonist phaclofen, but not by bicuculline. Moreover, pertussis toxin blocked the ability of (-)baclofen to inhibit the Ca^{2+}-activated ^{86}Rb-efflux, showing that GABA$_B$ receptor activation is mediated through the G-protein mechanism. Further, the activators of adenylate cyclase, forskolin, and phorbol ester also attenuated the action of (-)baclofen. These results suggest that the action of GABA$_B$ receptors involves G-protein and adenylate cyclase. This assay can be used to study GABA$_B$ receptor pharmacology.

520.11

AMINOALKYLINDOLE ANALGESICS: INHIBITION OF ADENYLATE CYCLASE IN RAT CEREBELLAR GRANULE CELLS. M. Pacheco, S.R. Childers and S.J. Ward. Depts. of Neuroscience and Pharmacology, Univ. Florida Coll. Med., Gainesville, FL 32610 and Sterling Res. Group, Rensselaer, NY 12144.

WIN 48098 (Pravadoline), an aminoalkylindole (AAI) analgesic, together with structurally related analogs devoid of cyclooxygenase activity, were previously shown to inhibit both basal and forskolin-stimulated adenylate cyclase (AC) activity in rat cerebellar membranes. This inhibition was GTP dependent, suggesting that AAI analgesics act through specific G-protein linked receptors in cerebellum to inhibit AC. Two other G_i-linked receptors, GABA(B) and adenosine A1, exist on cerebellar granule cells, and share common second messenger elements. In the present study, in cerebellar membranes, AAI agonists exhibited only partial additivity of AC inhibition with the GABA(B) agonist baclofen and the adenosine A1 agonist N6-(L-phenylisopropyl) adenosine (PIA). When agonists were added separately, maximal inhibition of AC was 30-40%. However, when any two of these agonists were added together, maximum inhibition was only slightly higher, at 40-50%. This lack of additivity of AC inhibition by different agonists suggests that baclofen, PIA and AAI agonists bind to different receptors sharing a common pool of G_i-proteins or AC catalytic units. The non-additivity also suggests that a population of AAI receptors is localized on cerebellar granule neurons. To test this hypothesis, effects of AAI agonists were determined on intracellular cAMP levels in primary cultures of cerebellar granule neurons from 8 day postnatal rats with an 8 day culture period. Forskolin (1 μM), in the presence of 0.5 mM IBMX, stimulated basal cAMP levels by 20-100-fold, while AAI agonists (1 μM) inhibited forskolin-stimulated cAMP levels by 30-90% in cerebellar granule neurons. The potency of AAI agonists were approx. 10-fold higher in whole cells as compared to isolated cerebellar membranes. Treatment of cells with pertussis toxin (100 ng/ml, 24 hr) blocked the AAI inhibition of forskolin-stimulated cAMP levels. These results demonstrate the presence of an AAI receptor-$G_{i/o}$ system localized on cerebellar granule neurons.

520.12

PHARMACOLOGICAL CONCENTRATIONS OF MELATONIN INHIBIT ADENYLATE CYCLASE ACTIVITY IN RAT BRAIN MEMBRANES. F. Hashemi* and L.P. Niles (SPON: E.S. Werstiuk). Dept. Biomedical Sciences, McMaster University, 1200 Main Street West, Hamilton, Ontario, L8N 3Z5.

Preincubation of rat cortical membranes with high concentrations of various benzodiazepines (BZs) has recently been reported to inhibit adenylate cyclase (AC) activity. The pineal indoleamine, melatonin, exhibits micromolar affinity for both central and peripheral BZ receptor sites, we have therefore, examined the effect of micromolar concentrations of this hormone on AC activity in rat brain. Preincubation of cortical membranes with melatonin (10-1000 μM) for 1 hour at 30°C caused an inhibition of forskolin-stimulated AC activity with an EC_{50} of about 200 μM. A maximal inhibition of about 30% was produced by 750 μM melatonin. Various receptor antagonists including metergoline, phentolamine and the central BZ receptor antagonist, Ro15-1788, did not block the effect of melatonin. Similarly, the inhibitory effect of diazepam was not blocked by Ro15-1788 suggesting the involvement of peripheral rather than central BZ receptors. Since melatonin also binds to peripheral BZ receptor sites, its pharmacological effects on AC activity may involve these sites. (Supported by the Ontario Mental Health Foundation and MRC, Canada.)

520.13

REVERSAL OF 8-BROMO-CAMP (8-B-CAMP)- AND NEUROTENSIN (NT)-INDUCED ATTENUATION OF DA INHIBITION OF DA NEURONS BY A PROTEIN KINASE (PK) A INHIBITOR, H8. W.X. Shi* and B.S. Bunney. Depts. of Psychiatry and Pharmacology, Yale University School of Medicine, New Haven, CT 06510.

We have previously reported that 8-b-cAMP, forskolin and IBMX mimic the action of NT; i.e., attenuate DA-induced inhibition of DA neurons. When administered concomitantly, IBMX potentiates NT's effects while SQ25536, an adenylate cyclase inhibitor, attenuates the effect of NT. These results suggest that NT may produce its effect by increasing intracellular cAMP, which in turn activates PK A. In order to determine whether PK A is involved in the action of NT, we have employed an inhibitor of PK A, H8. The spontaneous activity of DA cells was recorded extracellularly in brain slices. Bath application of H8 (80μM, for 20-30 mins) significantly or completely blocked the modulatory effects of both 8-b-cAMP and NT. Occasionally, H8 alone potentiated DA-induced inhibition. As H8 inhibits both PK A and C, the above effect may have been due to its effect on PK C. This possibility was tested by using H7, an analogue of H8, which is a more potent inhibitor of PK C than H8. In our preparation, treatment with H7 was found to be much less effective or to have no effect on NT's actions. These results not only suggest that cAMP and PK A can regulate the reponse of DA cells to autorecepor stimulation but also support the hypothesis that they may be involved in NT action.

520.14

PHORBOL ESTER INDUCED EFFECTS ON CAMP FORMATION IN A RAT HIPPOCAMPAL CELL LINE. Kelly A. Berg[1*], William P. Clarke[2], Ronald McKay[3] and Saul Maayani[1,2]. Depts. of Anesthesiology[1] and Pharmacology[2], Mount Sinai School of Medicine, CUNY, NY, NY 10029, and Dept. of Biology and Brain Science[3], MIT, Cambridge, MA 02139.

Initial steps of hormone-elicited signal transduction in excitable cells may be modulated by intracellular events that are initiated by coactivation of a second hormone receptor. For example, protein kinase C (PKC) mediated sensitization of adenylyl cyclase (AC) may be one aspect of interaction between the phosphatidylinositol and AC signalling systems. We are studying the effects of activation of PKC on forskolin (FRSK) and receptor-mediated cAMP production in a novel, neuronal cell line (HT4) derived from embryonic rat hippocampus.

Simultaneous application (30 min) of phorbol 12-myristate 13-acetate (PMA, 1 μM), with either Isoproterenol (ISO, 1 μM,beta-adrenoceptor), 5'-N-ethylcarboxamidoadenosine (NECA, 1 μM, Adenosine A_2 receptor) or FRSK (1 μM) amplified the formation of cAMP 3 -4 fold over FRSK or receptor stimulated cAMP formation alone (table) as determined by RIA. In addition, preliminary studies suggest that phenylephrine (alpha-1 adrenergic) and 5-HT (serotonergic) also enhance FRSK stimulation of cAMP in this cell line.

	BASAL	ISO	NECA	FRSK
- PMA	4.4 ± 0.3	94.1 ± 21.7	76.0 ± 11.1	51.2 ± 4.5
+ PMA	4.2 ± 0.6	355.8 ± 28.2	318.2 ± 120.4	161.4 ± 6.7

values are mean pmol cAMP/ mg protein ± SD; n=2

This neuronal cell line offers a useful model to study modulation of signal transduction processes in excitable cells.
(Supported by UPSH GM 34952 and NS 21991; WPC is a Revson fellow)

520.15

THE COUPLING OF CARDIAC-LIKE M2 MUSCARINIC RECEPTORS TO ADENYLATE CYCLASE IN THE LONGITUDINAL MUSCLE OF THE RAT ILEUM. L.M. Candell* and F.J. Ehlert (SPON: F. Leslie). Dept. Pharmacol., Univ. Calif. Irvine, Irvine, CA 92717.

The binding of the cardioselective muscarinic antagonist AF-DX 116 (11[[2-[(diethylamino)methyl]-1-piperidinyl]acetyl]-5,11-dihydro-6H-pyrido[2,3-b][1,4]benzodiazepine-6-one) was compared with its ability to interfere with muscarinic receptor-mediated inhibition of adenylate cyclase activity in the longitudinal muscle of the rat ileum. When measured by the competitive inhibition of the binding of the muscarinic antagonist [^{3}H]N-methylscopolamine ([^{3}H]NMS), the binding properties of AF-DX 116 were consistent with a two site model in which 72% of the receptors exhibited high affinity. The dissociation constants the high and low affinity sites (K_H and K_L) were estimated to be 0.078 and 2.3 μM, respectively. The highly efficacious muscarinic agonist oxotremorine-M caused a concentration-dependent inhibition of adenylate cyclase activity in homogenates of the longitudinal muscle of the ileum, with the maximal inhibition and the concentration of oxotremorine-M causing half-maximal inhibition (EC_{50}) being 31% and 0.46 μM, respectively. When measured by competitive antagonism of the adenylate cyclase response, the dissociation constant of AF-DX 116 (K_B) was estimated to be 0.14-.17 μM. Thus, there was good agreement between the K_B and K_H values of AF-DX 116, indicating that it is primarily the high affinity AF-DX 116 site which couples to adenylate cyclase in the longitudinal muscle of the rat ileum. *Supported by NIH Grant NS26511.*

520.16

An Inhibitor of cAMP-Dependent Protein Kinase Blocks the Activation of Rat Locus Coeruleus Cell Firing Induced by 8-Br-cAMP, Forskolin, and Ro20-1724. Y.-Y. Wang and G.K. Aghajanian, Depts. of Pharmacology and Psychiatry, Yale Univ. Sch. Med., New Haven, CT 06508

Previously, we found that the firing of noradrenergic neurons of the locus coeruleus (LC) was increased by agents that elevate cAMP levels (forskolin and Ro20-1724) or mimic cAMP action (e.g., 8-Br-cAMP). Here, we investigated whether the activation induced by above agents in LC neurons could be blocked by an inhibitor of cAMP-dependent protein kinase (PKI, Walsh inhibitor).

LC neurons in rat brain slices were recorded intracellularly with blunt electrodes (10-30 MΩ) filled with 2M KCl with or without PKI (100mg/ml; rabbit muscle, Sigma). PKI was ejected into neurons by passing negative current (0.6-2.0 nA) for 15-25 min.

Perfusion with 8-Br-cAMP (1 mM), forskolin (5 μM) or Ro20-1724 (100 μM) for 10 min increased the firing rate of LC neurons by ~2 fold when control electrodes were used. When PKI was ejected into LC neurons, the activation induced by 8-Br-cAMP, forskolin, or Ro20-1724 was attenuated by ~75%. Also, recovery was accelerated by PKI ejection and some cells hyperpolarized after discontinuing the cAMP-active agents. When a second dose of the above agents was administered following recovery, the increase in firing rate was further reduced or completely abolished by PKI.

Since the increase of LC cell firing produced by the cAMP-active agents was attenuated or blocked by PKI, our results suggest that this increase is mediated by a cAMP-dependent protein kinase.

520.17

EVIDENCE FOR AN ENDOGENOUS INHIBITOR OF ^{3}H-FORSKOLIN BINDING IN BRAIN. D. R. Gehlert, Experimental Therapeutic Branch, NINDS, NIH, Bethesda, MD 20892.

The compound forskolin is a potent activator of adenylate cyclase which is believed to activate via a direct interaction with the catalytic subunit. Binding studies using ^{3}H-forskolin have indicated that forskolin binding in the brain is stimulated by the interaction of Gpp(NH)p and NaF with Ns and the binding sites are found primarily in the basal ganglia. In order to determine if the catalytic unit of adenylate cyclase is regulated via the forskolin binding site in vivo, the present study was initiated to determine if an endogenous factor may interact with the forskolin binding site.

Rat brains were homogenized in an acidic solution and extracted twice with ether. The resulting solution was concentrated using vacuum centrifugation and pumped onto a semipreparative C-18 column and eluted with an acetonitrile gradient. Fractions which inhibited ^{3}H-forskolin binding to rat brain membranes were rechromatographed using additional reverse phase steps and the final active fraction separated using gel filtration chromatography. The inhibitory activity appeared to be proteins in the 30-50 KDa molecular weight range. The inhibition of binding was dose dependent and susceptible to protease digestion.

These results indicate that the binding of forskolin can be regulated by endogenous proteins in the brain.

DIFFERENTIATION, MORPHOGENESIS AND DEVELOPMENT: CYTOSKELETON

521.1

DIFFERENTIATION OF CAUDALMOST CELLS FROM REGENERATING SPINAL CORD IN VITRO. M.J.Anderson and D.L.Rossetto*. Dept. of Anatomy and Neurobiology, Colorado State Univ., Fort Collins, CO 80523.

Spinal cord of the teleost Apteronotus albifrons,unlike mammalian cord, can regenerate axons and produce new neurons after injury. This study characterizes the precursor cells and initial neuronal differentiation in vitro. Putative precursor cells were cultured from the caudalmost tip of regenerating spinal cords (L-15 medium with 10% fetal calf serum, on polylysine-coated plastic). After 7 das in vitro, the caudalmost cells are flat and polygonal or fibroblast-shaped. They do not exhibit thin, neurite-like extensions, and do not stain with monoclonal antibody against neurofilaments. After 14 das in vitro, cells in the caudalmost cultures begin to show altered morphologies, having more rounded somas and thin, spikey projections 5-100 μm long. Small lamellar areas at the tips of these projections may represent early growth cones. After 18 das in vitro, some of these cells stain with the anti-neurofilament antibody. These results suggest that: 1. cells from the caudalmost spinal cord can undergo differentiation over time in vitro, and 2. changes in morphology may precede the production of neuron-specific filaments during differentiation in vitro. Supported by NS-25951 and the College of Veterinary Medicine and Biomedical Sciences, Colorado State University.

521.2

ALTERED MAP2 DISTRIBUTION IN THE CYTOPLASM OF PURKINJE CELLS AND CA1 PYRAMIDAL CELLS OF THE REELER MUTANT MOUSE. P.R. Patrylo and R.S. Nowakowski, Department of Anatomy, UMDNJ-Robert Wood Johnson Medical School and Physiology/Neurobiology Program, Rutgers University, Piscataway, NJ 08854.

The distributions of microtubule associated proteins (MAP1 and MAP2) in the cerebellum and hippocampus of reeler (rl^{orl}/rl^{orl}) mice were studied by using indirect immunohistochemistry with monoclonal antibodies. MAP2 is localized in the dendrites but not the soma of every normally positioned Purkinje cell of the reeler cerebellum as well as in control littermates and C57BL/6J. In contrast, MAP2 is localized in both the soma and the dendrites of many of the nearby ectopic Purkinje cells in the central cell mass and the internal granule cell layer. In the reeler hippocampus MAP2 is localized in both the soma and dendrites of the CA1 pyramidal cells adjacent to the stratum radiatum and of all CA1 pyramidal cells of control littermates and C57BL/6J. In contrast, in the apical dendrites of the CA1 pyramidal cells adjacent to the stratum oriens MAP2 is decreased or absent. No differences in the cytoplasmic localization of MAP1 were observed.

Thus, in reeler mutants subsets of the Purkinje cells and the CA1 pyramidal cells have an altered MAP2 distribution. The fact that there is an increase in MAP2 in the soma of some of the ectopic Purkinje cells but a decrease in MAP2 in the apical dendrites of the CA1 pyramidal cells closest the stratum oriens indicates that the changes in MAP2 distribution are not a direct effect of the reeler mutation.

Supported by NIH (NS23647) and the Schizophrenia Research Program.

521.3

IMMUNOCYTOCHEMICAL STUDY OF HUMAN LENS EPITHELIUM S. MURAYAMA*, T. W. BOULDIN*, & K. SUZUKI. Dept of Pathology, Univ. Of North Carolina, Chapel Hill, NC, 27599-7525

The developmental and aging changes of the lens epithelium in humans from 14 weeks gestation to 81 years old were studied immunocytochemically in formalin-fixed paraffin-embedded postmortem material. Antibodies employed were anti-vimentin monoclonal antibody (Osborn, 1984) (1:10); anti-GFAP polyclonal antibody (DAKO) (1:200); and anti-ubiquitin monoclonal antibody (Mori et al, 1987) (1:10). 6-7 um thick sections were deparaffinized, incubated with the antibodies overnight at 4·C, and visualized with the avidin-biotin complex method. Normal lens epithelium showed constant immunoreactivity with anti-vimentin. Ubiquitin immunoreactivity appeared greatest in the early developmental period and decreased in old age. GFAP immunoreactivity appeared around 16 weeks gestation and plateaued around term. Two microphthalmic eyes and one from an anencephalic fetus showed lens epithelium that was immuno-reactive with anti-vimentin, but not with anti-ubiquitin or anti-GFAP. Our study indicated that there are different temporal patterns of expression of cytoskeletal proteins in developing lens epithelium and is in agreement with previous animal studies (Hatfield et al 1984). Ubiquitin immunocytochemistry may be a good tool to evaluate the function of human lens epithelium, as suggested by previous experimental studies (Jahngen et al 1986).

521.4

A MARKER GENE FOR DEVELOPING CNS SHOWS HOMOLOGY TO INTERMEDIATE FILAMENTS.
L.B. Zimmerman*, U. Lendahl* and R. McKay.
Depts. of Brain and Cognitive Science, Biology, Mass. Inst. of Technology, Cambridge, MA 02139, USA.

The Rat.401 protein is a 200 kD protein specifically found in neuronal stem cells in the rat CNS, but absent from adult CNS. Outside the developing CNS we find expression only in developing skeletal muscle.

Analysis of cDNA clones corresponding to the N-terminal portion of the Rat.401 protein identifies regions that are 30-50% homologous to intermediate filaments like desmin, neurofilament and vimentin at the amino acid level. These findings stimulated an investigation of the intracellular distribution of the Rat.401 protein with respect to other cytoskeletal components. Immunostaining of cells from the CNS precursor cell line ST15A reveals an intermediate filament-like distribution, but double-label experiments demonstrate that the Rat.401 protein does not colocalize with vimentin, nor with actin or tubulin. The intracellular distribution of Rat.401 protein is unaffected by treatment of the cells with colchicine and cytochalasin B.

Supported by grants from NIH, the PEW Foundation, the Rita Allen Foundation and an EMBO Fellowship to U.L.

521.5

THE EXPRESSION OF THE NEURONAL INTERMEDIATE FILAMENT PROTEIN PERIPHERIN IN THE DEVELOPING RAT EMBRYO. J.D. Gorham* and E.B. Ziff* (SPON: J. Jacoby). Dept. of Biochem. and Kaplan Cancer Ctr., New York Univ. Med. Ctr., New York, NY 10016.

The cDNA coding for the type III intermediate filament protein (IFP) peripherin (per) was originally identified as a NGF inducible mRNA in PC12 cells. Previous *in situ* hybridization (*ish*) studies in the rat localized expression of this mRNA to all peripheral neurons, ventral motoneurons, and a small subset of nuclei in the brain (Leonard, et al., 1988). In the current study, the expression of per mRNA was examined in the spinal cord and PNS of the rat embryo at various stages of development. Sprague-Dawley rat embryos were processed for *ish* and probed with ^{35}S-labeled per cRNA. Expression was seen in sympathetic ganglia (SG), dorsal root ganglia (DRG), and ventral motoneurons (VM) at gestation days E13.5 and E15.5, but not at day E10.5. At gestation day E15.5, expression was also seen in enteric neurons. To extend the above studies, a polyclonal antibody against a bacterially expressed trpE/per fusion protein was generated in rabbits. Serum was affinity purified and assayed for specificity. Anti-per recognizes in total PC12 extracts a single 58 kDa protein which is induced by NGF and is enriched in the cytoskeletal fraction; antiper shows no cross-reactivity with other IFP's. The per protein is expressed in both naive and NGF treated PC12 cells. In differentiated PC12 cells, per is found in the cell soma and in neurites. Application of antiper to embryo sections recapitulated the distribution seen with the mRNA: fluorescence was seen in SG, DRG, and VM at ages E12.5 and E13.5 and in axons emanating from these structures. Expression was not seen in an embryo of age E11.5, further establishing the time of initial expression. For both the *ish* and immunofluorescence studies, per expression was never seen in regions containing dividing or migrating neuroblasts. We conclude that per is expressed in the developing rat in peripheral neurons and in ventral motoneurons only after these cells have migrated to their final destinations; expression thus correlates with neurite outgrowth.

521.6

REORGANIZATION OF NEURONAL CYTOSKELETON FOLLOWING STIMULATION WITH EXCITATORY AMINOACIDS. (SPON: Brain Research Association). D. Bigot[1], A. Matus[2], and S.P. Hunt[1]. MRC Molecular Neurobiology Unit[1], Cambridge CB2 2QH, UK; Friedrich Miescher Institute[2], 4002 Basel, CH.

Functional change in the nervous system and aspects of certain neurodegenerative diseases may involve modification of the neuronal cytoskeleton. Here we show that stimulation of cortical neurons *in vitro* with selective glutamate agonists results in the rapid reorganization of the cytoskeleton. Cortical neurons (from E18-20 rat embryos) were plated onto astrocytic glial beds and maintained in serum-free medium. After 14 days in culture neurons were stimulated with EAA for periods of 5-120', fixed after 2 h and MAP2, tau or tubulin visualised by fluorescent immunohistochemistry and confocal laser microscopy. Stimulation with quisqualate (10-50 uM) or kainate (50-100 uM) but not NMDA or K$^+$ (50 mM) resulted in both an increase in the amount of perikaryal and neurite staining and a reorganization of the diffuse cell body labelling into a distinctive network of filamentous staining continuous with the neuritic cytoskeleton. This occurred in 40% of cortical neurons, was blocked by CNQX but not pertussis toxin, cycloheximide, or actinomycin. Prior treatment of cultures with quisqualate prevented depolymerization with nocodazole and the effect was independent of external calcium, but dependent on sodium and partially depressed by chloride removal. In contrast, NMDA but not quisqualate or kainate caused an increase of diffuse tau staining in neuronal cell bodies. This suggests that the neuronal cytoskeleton can be differentially regulated by EAAs.

521.7

REGIONAL DIFFERENCES IN THE TIMING OF PURKINJE CELL DENDRITIC OUTGROWTH DEMONSTRATED BY THE IMMUNOSTAINING PATTERN OF MICROTUBULE-ASSOCIATED PROTEIN 2 (MAP2). K.M. Hamre, C.R.Goodlett, and J.R. West. Dept. of Anat., Univ. of Iowa, Iowa City, IA 52242.

Microtubule-associated protein 2 (MAP2) has been proposed to play a role in the formation of dendrites and maintenance of neuronal shape. This study examined whether developmental changes of the Purkinje cell dendritic trees could be monitored using an antibody against MAP2. Sections from the cerebellar vermis of developing rat pups from postnatal day 1 (PD 1: 24 hours after birth) to PD 12 were processed for immunocytochemistry with an anti-MAP2 antibody, using the peroxidase-antiperoxidase method. On PD 1, MAP2-stained Purkinje cell somas were aligned in multilayered rows, and possessed only small, short processes. Starting around PD 3, the Purkinje cells began to be aligned in a monolayer and extrasomal processes could be seen extending from the cells in multiple directions. Importantly, differences both among lobules and within lobules in the timing of dendritic outgrowth were clearly evident. As early as PD 4 in lobule 9, a primary dendritic branch could be identified, followed by progressive increases with age in the branching and complexity of dendritic trees. A comparison of dendritic development in lobules IX and X with that of lobules VI and VII, indicates that the Purkinje cells in IX and X initiate dendritic branching 3 to 4 days before those in lobules VI and VII. Within lobules, particularly within V, VI and IX, the dendrites of Purkinje cells in the proximal portion of the lobule develop sooner than those in the distal portion. This study demonstrates that Purkinje cells exhibit regional and temporal differences in dendritic development, and that the immunostaining pattern of MAP2 is an effective way of characterizing these differences. (Supported by grants AA05523 and AA07313. We thank Dr. R. Vallee for the MAP2 antibody.)

BIOLOGICAL RHYTHMS AND SLEEP: OTHER IV

522.1

THE CIRCADIAN RHYTHMS OF OBESE AND LEAN ZUCKER RATS. D. M. Murakami,* B. A. Horwitz, J. S. Stern[1] and C. A. Fuller (SPON: F. Gorin). Department of Animal Physiology and Department of Nutrition[1], University of California, Davis, CA 95616.

The circadian system plays an important role in the neural contol of feeding and metabolism, and may be involved in the abnormalities exhibited in the obese condition of fatty Zucker rats. Therefore, this study examined the circadian rhythms of body temperature (Tb) and activity in fatty and lean Zucker rats. Fatty and lean littermates were implanted with biotelemetry units (Minimitter) in order to record Tb and activity at 10 minute intervals. The rats were placed in individual cages and sequentially exposed to three lighting conditions (LD 12:12, LL and DD, L at 400 lux). In all three light conditions, there was not a significant difference in mean Tb between fatty and lean rats. However, the mean activity of fatty rats was significantly lower than that of lean rats. In terms of circadian parameters the circadian amplitude of Tb and activity was significantly depressed in fatty rats compared to that of leans in LD. However, the circadian amplitude of Tb did not remain significantly different in LL or DD. The circadian amplitude of activity remained significantly different in both LL and DD. In addition, the freerunning period during DD was significantly longer in fatty rats compared to lean controls. Such circadian differences may reflect the neurophysiological changes associated with the genetic obesity.

522.2

CIRCADIAN PHASE CHANGES RESPONSE TO HOMEOSTATIC CHALLENGE AND EAT-DRINK RATIOS IN RATS. R.F. Johnson and A.K. Johnson. Depts. of Psychology, Pharmacology and the Cardiovascular Center, Univ. of Iowa, Iowa City, IA 52242.

We have reported differential homeostatic drinking at two phases (early light vs. early dark). The response is dose-dependent but not dependent on the type of challenge or different illumination (RFJ & AKJ, SNS Abstr., 14:1298, 1988).

To obtain homeostatic drinking across the circadian day, 10 rats were allowed to freerun and were injected with hypertonic saline at 3-4 day intervals. Quantity of water intake to this challenge was monitored for 3 hrs in the absence of food. To examine evidence of circadian modulation of drinking in response to food intake, 10 other rat's eating and drinking were continuously recorded while the rats were on an LD cycle. Meals and meal-associated drinking were defined using set criteria, and the amount drank was divided by meal size (drink-eat ratio). Data from both studies were converted to standard deviations from individual means, expressed in circadian time, and put into 2-hr bins.

Both the drink-eat ratios and the quantity of homeostatic drinking displayed prominent rhythms (low in inactive and high in active phase). This suggests a circadian modulation of both homeostatic and meal-induced drinking.

522.3

SEX DIFFERENCES IN THE PATTERN OF LIPID DEPLETION FROM ADIPOSE TISSUE IN SHORT DAY-EXPOSED SIBERIAN HAMSTERS. T. J. Bartness. Depts. of Psychology & Biology, Georgia State University, Atlanta, GA 30303.

Siberian hamsters exposed to short days decrease their body weight, an effect reflected nearly exclusively as a decrease in carcass lipid. We previously reported that internally-located white adipose tissue (WAT) fat pads (*e.g.*, retroperitoneal WAT [RWAT]) were depleted of lipid at a faster rate than more externally-located WAT pads (*e.g.*, inguinal subcutaneous WAT [IWAT]) following 6 or 12 wks of exposure to short days in male hamsters. In the present experiments, male and female hamsters were housed in long days (LD 16:8) or transferred to short days (LD 8:16) for 6 wks. Short day-exposed hamsters of both sexes had decreased body weight and carcass lipid content; however, relative to their long day controls, short day-housed male hamsters had greater depletions of lipid from RWAT than IWAT, and this was reflected as a disproportionate decrease in RWAT fat cell size, with no effect on fat cell number (FCN). In contrast, female hamsters had uniform decreases in IWAT and RWAT mass, reflected as uniform decreases in fat cell size, without affecting FCN, relative to their long day controls. These results suggest that gonadal steroids may differentially modify the responses of adipocytes to changes in daylength in Siberian hamsters in a fat pad-specific manner. Supported by NIH DK-35254.

522.4

ABDOMINAL VAGOTOMY BLOCKS HYPERPHAGIA AND BODY WEIGHT GAIN IN SYRIAN HAMSTERS EXPOSED TO SHORT PHOTOPERIOD (SP). M.O. Miceli, C.A. Post* and Z. Woskowska*. Depts. of Psychiat. & Biomed. Sci., McMaster Univ. & St. Joseph's Hosp. Res. Inst., Hamilton, Ontario, Canada L8N 4A6.

Syrian hamsters housed under SP conditions increase their food intake (FI) and body weight (BW). To determine possible vagus nerve involvement in these responses to SP, we examined FI and BW in groups of vagotomized (VAGX; n's=10) and sham operated (SHAMS; n's=8) hamsters maintained under a long photoperiod (LP; L:D 14:10) or a SP (L:D 8:16). Three days after surgery, hamsters were provided a high fat diet (crisco & chow) and housed under an assigned photoperiod. All animals gained weight upon the availability of the high fat diet. However, FI and BW gains were greater in SHAMS maintained under SP than in SHAMS housed under LP. FI and BW gains in VAGX hamsters maintained under LP and SP were not different than in SHAMS housed under LP. Estrous cyclicity during the 7th-10th week of the study was evident (from vaginal discharges) in all SHAMS and VAGX hamsters in LP, but not in any of their counterparts in SP. Our results indicate that the vagus is not important for the development of dietary obesity, but is critical for the expression of elevated FI and BW under SP. Our findings with VAGX also suggest that separate mechanisms underly seasonal changes in BW regulation and reproductive function. We are now examining whether VAGX inhibition of SP-induced hyperphagia and obesity results from blockade of vagally-mediated hyperinsulinemic responses to SP.

522.5

ONE LONG DAY STIMULATES GONADAL GROWTH IN YOUNG SIBERIAN HAMSTERS. C.S. Whaling,* C.M. Finley,* N. Spears,* and I. Zucker (SPON: G. Eskes) Psych. Dept., Univ. of Calif. Berkeley, CA 94720.

Siberian hamsters undergo morphological changes that are controlled by day length. Testes undergo regression, uterine weight, and total body weight are reduced in short day lengths. Hamsters do not, however, respond to short day lengths in the expected manner if they have been exposed to one extended long day (33 h of continuous light) at weaning (Spears et al., in prep.). We tested whether a 4 h extension of the light phase on the day of weaning exerts long term effects on the reproductive axis.

Hamsters were maintained in a 16L:8D photoperiod to 18 days of age. On day 18 the light period was extended by 4 h for the experimental group only. On day 19 experimental and control hamsters were transferred to a short photoperiod (8L:16D). Experimental males had significantly heavier gonads than control animals at 30 (480 ± 22 vs 306 ± 44 mg) and 35 (553 ± 68 vs 158 ± 48 mg) days of age. Uteri were heavier in experimental than control females at 30 but not 35 days of age. An increase in photoperiod duration at the time of weaning has long term photostimulatory effects on the reproductive system.

Supported by NIH Grant HD 02982.

522.6

PHOTOSTIMULATION OF THE REPRODUCTIVE AXIS BY A SINGLE LONG DAY IN SIBERIAN HAMSTERS IS NOT CONTINGENT UPON PHASE SHIFTING OF ENTRAINED CIRCADIAN RHYTHMS. C.M. Finley*, C.S. Whaling*, N.R. Spears* and I. Zucker, Dept. of Psychology, Univ. of California, Berkeley, CA 94720.

Siberian hamsters given a single long day (LD) at weaning and housed thereafter in short days (SD) have large gonads at day 35. The photostimulatory effect induced by one LD at weaning might be mediated by phase shifting of circadian rhythms and dependent on subsequent changes in entrainment to SD photoperiod. To test this hypothesis, animals gestated and lactated in SD were weaned at 18 days and given either an additional SD (controls) or an extended LD (experimentals). Thereafter all hamsters were maintained in constant darkness. At day 35 experimental animals had significantly heavier testes (214.2 ± 31 vs 20.8 ± 3 mg) and uteri (19.4 ± 2 vs 10.5 ± .4 mg) than did controls. Because animals in constant darkness were not entrained to a SD light dark cycle, changes in phase angles of entrainment of circadian rhythms to the SD photoperiod are not necessary and are not likely to be responsible for the original phenomenon. Supported by GRANT HD-02982.

522.7

TIMED MELATONIN INFUSIONS INDUCE UTERINE REGRESSION IN PINEALECTOMIZED SYRIAN HAMSTERS. M. H. Brown and G. N. Wade. Dept. of Psychol., Univ. of Massachusetts, Amherst, MA 01003.

Infusions of melatonin (MEL) that mimic the short day duration of endogenous MEL secretion cause reproductive involution in pinealectomized Siberian hamsters regardless of time of day of administration (e.g., Am. J. Physiol. 255:R812, 1988). The following is a report of research in progress investigating the response of Syrian hamsters to infusions of MEL. Female hamsters were housed in long days (16L:8D), pinealectomized, and divided into groups of 8-9 each. Daily infusions of MEL (100 ng/day) or saline (SAL) were delivered via subcutaneous cannulae. In experiment 1 MEL and SAL infusions 12 hrs in duration, begining 4 hrs before lights off, were given for 7 wks. In experiment 2, MEL infusions of 11, 8, and 5 hrs and SAL infusions of 11 hrs duration were given for 10 wks. The midpoint of these infusions corresponded with the midpoint of the dark phase of the light-dark cycle. While 15 of 16 control hamsters maintained estrous cyclicity (indicated by post-ovulatory vaginal discharge) throughout the experiment; 3 of 8 hamsters that received 12-hr, 3 of 8 that received 11-hr and 1 of 8 that received 8-hr infusions of MEL became anestrous. Furthermore, 11-, and 12-hr MEL infusions resulted in statistically significantly reduced mean uterine weight (compared to 11- and 12-hr SAL infusions, respectively). Thus, long but not short duration MEL infusions cause uterine regression in Syrian hamsters.

Supported by NS 10873, DK 32976, MH 00321, and MH 09564.

522.8

THE PARAVENTRICULAR NUCLEI (PVN) INFLUENCE DAILY TORPOR IN SIBERIAN HAMSTERS. N.F. Ruby and I. Zucker. Dept. of Psychology, University of California, Berkeley, CA 94720.

The role of the PVN in mediating daily torpor was studied in adult hamsters maintained in a short photoperiod (8 h light/day). Animals with radiofrequency transmitters were maintained in 15°C and body temperature (T_b) recorded at 10 minute intervals using a telemetry system beginning 8 weeks after initial short day exposure. Animals manifesting at least 4 torpor bouts (T_b < 30°C) in a 14 day interval received lesions of the PVN (PVNx) or sham lesions; hamsters with neural damage outside the PVN constituted a separate group. Ablation of the PVN completely terminated the expression of torpor in 60% of animals tested, and had no effect in the remaining PVNx hamsters. Torpor was unaffected in sham-operated animals and in those with partial damage to the PVN and other neural structures.

Ablation of the suprachiasmatic nuclei (SCN) terminates the expression of torpor while pinealectomy does not. Thus, elimination of torpor in PVNx hamsters is not due to denervation of the pineal gland. These results suggest SCN efferents that course through the PVN, or the PVN itself may be part of a neural substrate for expression of torpor.

522.9

ABNORMAL TEMPERATURE VARIATIONS IN HUMAN EATING DISORDERS. J.K. Nishita, E.H. Ellinwood, Jr., W.J.K. Rockwell*, M.C. Kasper*, D.C-W. Hsu*, and M-M. Taylor*. Dept. of Psychiatry, Duke Univ. Med. Cntr., Durham, NC 27710.

Anorexia nervosa and bulimia nervosa are psychiatric illnesses which manifest prolonged or permanent disturbances in metabolic and hypothalamic function. Evidence for cyclical abnormalities in endocrine, sleep, and thermoregulatory rhythms have been reported. We attempted to describe patterns in oral temperature (OT) rhythmicity that may reveal concurrent neuroendocrine deficits in eating disorder (ED) patients. We report the occurrence of chronic hypothermia and describe cyclical patterns of OT in 20 patients. By measuring OT at five different times daily 13 patients showed chronic hypothermia (OT<36.1°C) or unstable OT. Most patients showed hyperreactivity to ambient cold and to a cold pressor test (CPT), marked changes in plasma AVP and NE when in the cold, extreme variations in OT (2-3°C) at room temperature, irregular or displaced diurnal OT cycles, and a variety of differences in chronobiology cosinor parameters (acrophase, amplitude, and mesor). Unstable OT correlated with CPT-induced heat release (r=0.71) and hypothermia (r=0.76).

Persistence of abnormal diurnal thermoregulation rhythmicity may result from an underlying neuroendocrine imbalance in ED patients following self-starvation/malnutrition.

522.10

PHASE-TYPING SEASONAL AFFECTIVE DISORDER USING A CONSTANT ROUTINE K. Dahl, D. Avery, M. Savage, G. Brengelmann, L. Larson, M. Vitiello, & P. Prinz. University of Washington, Seattle, Wa 98195. (SPON: M.D. Leibowitz)

The phase of the circadian rhythm in patients suffering from Seasonal Affective Disorder (SAD) was assessed using a constant routine to "unmask" the endogenous circadian rhythm by controlling variables influencing core body temperature. Subject group consisted of 10 female hypersomnic SADs and 9 controls. Eight SADs were restudied after responding to AM bright light. During 27 hours in the hospital subjects were sleep-deprived at bed rest with light (60 lux) and temperature held constant. Sweat rates, rectal and skin temperatures, were continuously monitored. Blood samples for NE, EPI, cortisol, TSH, and melatonin were drawn every hour. The temperature data were analyzed by cosinor analysis. The groups were compared with one-tailed and two-tailed unpaired and paired t-tests. The rectal temperature acrophase of the SAD subjects was significantly ($p<.05$) phase-delayed relative to controls (17:36 vs 15:44). There was a nonsignificant trend (one-tailed $p=.09$) for the morning bright light treatment to phase-advance the acrophase (17:41 to 16:20). The mean temperature amplitudes of the SADs and controls were not significantly different (.42°C vs .48°C) and did not change with light treatment (.42°C vs .42°C).

522.11

OLFACTORY BULBECTOMY INCREASES BODY TEMPERATURE AND ALTERS DAILY TEMPERATURE RHYTHM IN MICE. A.R. Lumia*, B. Possidente* and H. Herz* (SPON: M. McGinnis). Biopsychology Program, Skidmore College, Saratoga Springs, NY 12866.

Bulbectomized rodents provide an animal model for agitated depression (Lumia et al. Soc. Neurosci. Abst., Vol. 14, p. 908, 1988; Jesberger, J.A. and Richardson, J.S., Behav. Neurosci. 100:256-274, 1986). We have previously shown that bilateral olfactory bulbectomy (OBX) alters the period, phase and amplitude of circadian activity rhythms in mice (Lumia et al., 1988) and rats (Lumia et al., Soc. Neurosci. Abst., Vol. 12, 1987) similar to changes reported for human geriatric depressives (Teicher et al., Arch. Gen. Psych. 45:913-917, 1988). Here we show that OBX mice have an altered daily rhythm for temperature in a 12:12 LD cycle, and a higher mean temperature (36.29 +/- 0.32 for OBX mice vs. 34.95 +/- 0.40 in controls). The controls show a typical nocturnal peak temperature which drops back to the 24-hour mean at the end of the dark period and reaches a low point for the cycle one hour after the lights go on. The OBX mice show a similar peak, but remain elevated for two hours into the light period and reach a low point three hours later than controls.

522.12

LOCOMOTOR ACTIVITY ALTERS THE CIRCADIAN RHYTHM OF WHEEL-RUNNING IN GOLDEN HAMSTERS. J.S. Kruse. University of Rochester School of Medicine and Dentistry, Rochester, NY 14642.

Running-wheel activity is commonly used to assay the output of central circadian pacemakers. In particular, Golden hamster locomotion is frequently studied because of the precision and reliability of wheel-running rhythms in these rodents. The experiment presented here relied on locomotion as a marker for the activity of internal clocks, but was designed to demonstrate that locomotor activity *per se* can influence circadian oscillators. Male Golden hamsters kept in constant darkness had free running wheel-running rhythms significantly greater than 24 hours (24.35 hours) during a 4 week baseline interval. For the next 10 days under constant darkness, wheels were locked for 23 hours each day in an attempt to entrain activity to a 24 hour period. All hamsters ran in the wheels during the one hour of daily access. Upon release from entrainment, with constant access to the wheels and constant darkness, free running wheel-running rhythms resumed with a period significantly less than during the baseline (23.90 hours). The phase of running-wheel onset was closer to the phase during the entraining interval than to the phase extrapolated from the baseline interval (1.1 vs. 6.5 hours). Thus, a scheduled pattern of daily running was able to fundamentally alter subsequent running rhythms. Further investigations will look at concomitant changes in other behavioral circadian rhythms during restricted running regimens.

522.13

Circadian Activity Characteristics and Light-Responsivity of Hooded Rats. Mark Bauer. Dept. Psychiatry, Univ. Pennsylvania, Philadelphia, PA 19104

Intensity and precision of circadian wheel-running patterns were evaluated in 3 commonly available outbred rat strains, Long-Evans hooded (LE), Wistar (W), and Sprague-Dawley (SD). Intensity of wheel-running (wheel revolutions per day) significantly differed among strains under both entrained conditions ($F=8.9$, with LE=W>SD) and in constant darkness ($F=14.7$, with LE>>W=SD), without difference among strains in precision of running onset in constant darkness ($F=0.17$). In LE, initial studies of phase-delays in response to brief (5') white light pulses showed the expected dependence of magnitude of phase-delay on light irradiance ($96\pm35'$ [mean $\pm$ s.d.] @ 0.46 uW/cm^2 vs. $157.1\pm35'$ @ 505 uW/cm^2; $p<0.007$). While there was good correlation ($r=0.71$) between magnitude of phase-delays after exposure of the same animal to 2 identical bright light pulses at a 3-week intervals, the second phase-delay was significantly larger than the first ($135\pm44'$ vs. $115\pm37'$, $p<0.05$) suggesting that caution must be used in interpreting results based on exposure of the same animal to multiple light pulses, at least in this paradigm.

522.14

EFFECTS OF FORCED TREADMILL RUNNING ON CIRCADIAN RHYTHMS IN THE RAT. R.Mistlberger. Dept. Psych. Simon Fraser University, Burnaby BC Canada.

Scheduled wheel running can entrain or phase shift circadian rhythms of hamsters (Mrosovski, 1986). Appropriately timed exercise may thus have value in reducing jet lag. However, observations in the rat cast some doubt on the species generality of these findings. Rats fed a single daily meal show intense food anticipatory wheel running, but this rarely entrains their free-running rhythms. In the present study, 18 male rats in dim light were run on a treadmill for 2 h daily for 35 days. This procedure did not entrain the free-running rhythm in any rat, including those with tau within 20-30 min of 24 h, nor did it result in anticipatory activity to the daily session. However, one rat showed a large phase delay when the treadmill session occurred at CT22 and several rats showed subtle period changes that may reflect relative coordination. These results suggest that timed activity may effect the circadian system of rats but that this effect is far weaker than that observed in hams ters. Not surprisingly, attempts to alter the rate at which rats reentrain to shifted light cycles using timed treadmill running have to date been inneffectual.

522.15

A FUNCTIONAL RUNNING WHEEL IS NOT NECESSARY FOR THE DEVELOPMENT OF SPLIT LOCOMOTION IN SYRIAN HAMSTERS (MESOCRICETUS AURATUS). D.C. Beretta* and C.E. McCormack. The Chicago Medical School, North Chicago, IL 60064.

The fact that in continuous light (LL) the locomotor rhythm of the Syrian hamster splits into two distinct components, each of which briefly displays a different period (tau), comprises evidence that the circadian oscillator is composed of multiple coupled oscillators. However, since rotation of the running wheel by the hamster can, in itself, alter the phase of the locomotor rhythm (Nature 330:372, 1987), it is possible that "splitting" in LL represents a wheel-induced artifact rather than a manifestation of multiple oscillators. Thirty-six, 75-day-old male hamsters (Charles River, LVG) were exposed to 300 lux of cool white fluorescent LL, and Wahman wheel-running activity in individually isolated chambers was recorded. After one week, the wheels of 18 experimental (exp) hamsters were locked, thus preventing wheel rotation even though each animal could freely enter and exit its wheel. After an additional 53 days in LL, the wheels were unlocked and within four days, 8/18 of the exp. hamsters developed split locomotion, some on the first day after unlocking. Splitting occurred in 9/18 controls (wheels never locked) kept in LL for the same length of time, but never before 6 weeks in LL. Tau of locomotion, measured during the second week after unlocking, did not differ significantly between exp. and control groups whether locomotion was split (24.19 ± .05 h vs 24.21 ± .03 h) or unsplit (24.60 h ± .09 vs 24.71 h ± .05) (x ± SEM). These results demonstrate that a functional running wheel is not essential for splitting to develop; thus splitting is not a wheel-induced artifact. Supported by BRSG S07 RR05266-27.

522.16

THE EFFECTS OF ACUTE INCREASES IN ACTIVITY ON THE CIRCADIAN CLOCK OF HAMSTERS. C. Wickland and F. Turek. Dept. of Neurobiology & Physiology, Northwestern Univ., Evanston, IL 60208.

Injections of the short-acting benzodiazepine, triazolam (Tz), are associated with acute increases in activity and phase dependent shifts in the circadian rhythm of locomotor activity in golden hamsters. In order to investigate whether the phase shifts in the hamster's rhythm of activity induced by Tz are mediated by the increased activity, "Minimitter" transmitters were used to measure the total locomotor activity of hamsters in response to "pulses" of a running wheel. Hamsters housed in constant light in cages without running wheels were given a new cage once every 2 weeks at 1 of 8 different circadian time points. Half of the cages contained a wheel that was removed after 1 hour and half of the cages contained no wheel. The phase response curve (PRC) derived from the wheel "pulses" has a shape similar to the PRC generated by injections of Tz. Phase advances in the activity rhythm occur at CT's 6 and 9, and delays occur at CT's 0 and 3. The only significant phase shifts induced by transfer to new cages without wheels were phase delays at CT 0. Although presentations of new cages with or without wheels resulted in increased activity 97% of the time, no significant correlation was found within a group between the increase in activity and the magnitude of the phase shift. These results indicate that presentation of a novel stimulus which increases activity can affect the circadian clock in a manner similar to that for Tz; however, the amount of increase in activity is not directly related to the magnitude or direction of the phase shift.

DIFFERENTIATION, MORPHOGENESIS AND DEVELOPMENT: MOLECULAR CORRELATES

523.1

THE SHITHEAD EXPERIMENTS: VISCERAL, BUT NOT CUTANEOUS TARGETS, DICTATE CALCITONIN GENE-RELATED PEPTIDE (CGRP) ENRICHMENT IN TRIGEMINAL GANGLION AFFERENTS. K. Horgan and D. van der Kooy, Dept of Anatomy, University of Toronto, Toronto, Ontario, Canada, M5S 1A8.

The neurotransmitter CGRP is present in 25.5% of adult trigeminal ganglion sensory afferents projecting to a visceral target, the cerebral arteries.This CGRP enrichment contrasts with only 10% CGRP positive cells in the population of trigeminal ganglion afferents projecting to a cutaneous target, the forehead skin. Another visceral target, the stomach antrum, is massively enriched in CGRP containing afferents from dorsal root ganglia (DRG) - approximately 75-85% of DRG afferents to stomach antrum are CGRP positive. To test if visceral targets induce CGRP enrichment in trigeminal afferents, fetal stomach antrum tissue was transplanted into the path of the developing cutaneous trigeminal projection to the forehead skin in postnatal day (PND) 0 rat pups. On PND 30, the retrograde tracer True Blue was applied in controls to label the trigeminal projection to the forehead skin and in transplant animals to label the equivalent trigeminal projection now innervating stomach antrum. CGRP was present in 24.3±3.4% of retrogradely labeled cells in transplant animals and in 12.5±1.4% of labeled cells in control animals. The number of True Blue labeled cells in transplant animals, 587.3±18.7, was not significantly different from the number of labeled cells in controls, 539.3±134.8, indicating that transplanted stomach antrum tissue respecifies non-CGRP afferent nerves to the CGRP phenotype. These results suggest that visceral targets actively induce CGRP enrichment in trigeminal sensory afferent projections. However, the CGRP enrichment (25%) in the trigeminal projection to transplanted stomach antrum is low relative to the 75-85% CGRP positive DRG afferents projecting to stomach antrum in vivo, but is comparable to the CGRP enrichment seen in the trigeminal projection to the cerebral arteries. This suggests the presence of an intrinsic upper limit to CGRP enrichment in trigeminal ganglion projections.

523.2

EXPRESSION OF THE PRODYNORPHIN GENE DURING THE DEVELOPMENT OF THE RAT CEREBRAL CORTEX. AN "IN SITU" HYBRIDIZATION STUDY. G. Alvarez-Bolado*, A. Fairén*, J. Douglass and J.R. Naranjo* (SPON: ENA). Instituto Cajal, CSIC, 28006 Madrid, Spain, and Institute for Advanced Biomedical Research, Oregon Health Sciences University, Portland, OR 89230.

A population of cortical neurons contains the opioid peptide dynorphin; the laminar distribution of these neurons and their developmental patterns are not well known. We have utilized a riboprobe derived from cDNA for rat prodynorphin (plasmid SP64D1.7; Civelli et al., PNAS 82:4291, 1985) to localize dynorphin neurons. Rats aged from E15 through P15 were used. Prenatal animals did not show any labeling in the cerebral cortex. At P4, prodynorphin was expressed in a small number of cortical neurons for the first time. The autoradiographic signal was restricted to perikarya. Labeled neurons were located in the interhemispheric cortex and in neocortical areas; no labeled neurons were observed below the rhinal fissure. A progressive increase in the numbers of neurons expressing prodynorphin mRNA was observed until P15. Labeled cells were located in layers II-V, although layer IV showed the lowest numbers. Layer VI contained no labeled neurons at any stage. Areal distribution was maintained during the period analyzed. Controls: slices pretreated with RNase showed no autoradiographic signal; parallel studies using GAD riboprobes (plasmid SP65-13; Kaufman et al., Science 232:1138, 1986) revealed different spatio-temporal patterns of labeling.

523.3

TRANSPLANTATION AND MOLECULAR ANALYSIS OF INTRINSIC VERSUS ENVIRONMENTAL REGULATION OF CEREBRAL CORTICAL PHENOTYPE. M.Barbe*, P.Levitt. Dept. of Anatomy, Medical College of Pennsylvania, Phila., Pa. 19129.

Limbic system associated membrane protein (LAMP) is expressed early in cerebral cortical development. We used LAMP expression as a specific marker of limbic cortical neuron phenotype to evaluate intrinsic and extrinsic regulation of commitment of cortical neurons to limbic or nonlimbic lineages. Limbic and non-limbic cortical areas after LAMP expression begins (E18) were excised from either rhinal sulcus or presumptive somatosensory cortex, respectively. Slabs labeled with 0.002% Fast Blue were transplanted into a small lesion cavity in P2 host perirhinal or somatosensory cortex. Animals were sacrificed 2 weeks later and analyzed immunohistochemically for LAMP expression. Total number of surviving transplanted cells (Fast Blue) and those double labeled for Fast Blue and LAMP were counted. Percentages of double labeled cells from perirhinal cortex transplanted into somatosensory cortex are- large neurons: 62%, not classified: 32%, glia or endothelial cells: 0%. Perirhinal cortex transplanted into perirhinal cortex- large neurons: 74%, not classified: 45%, glia or endothelial cells: 0%. In striking contrast, there were no double labeled cells in transplants from somatosensory cortex into host somatosensory or perirhinal cortex. The same experiment was performed using E14 cortex, a developmental period before LAMP is expressed, and gave similar results. These data suggest that specification of cortical areas, based on molecular phenotype, may be regulated intrinsically rather than by local environment. Supported by March of Dimes Grant 1-919 and NIH Fellowship NSO7287.

523.4

IN SITU HYBRIDIZATION ANALYSIS OF THE DEVELOPMENT OF DARPP-32 mRNA IN RAT STRIATUM. E.L. Gustafson, M. Ehrlich, P. Trivedi*, T. Kurihara* and P. Greengard. Lab. of Molecular and Cellular Neuroscience, The Rockefeller Univ., New York, NY 10021.

The development of mRNA encoding DARPP-32 (dopamine- and cAMP-regulated phosphoprotein) was investigated in the rat striatum by in situ hybridization histochemistry, from embryonic day 19 to adult. This was accomplished using an ^{35}S-labeled oligonucleotide probe complementary to rat DARPP-32 mRNA, the specificity of which was established by Northern blot and hybrid melting temperature analyses. In the caudate-putamen (CP), DARPP-32 mRNA was first detected on the day of birth (P0), which contrasts with the first immunocytochemical detection of the protein on embryonic day 17 (Foster et al., J.Neurosci., 7:1994, 1987). DARPP-32 mRNA levels in medium spiny neurons of the CP increased markedly from P0 to P16. At P16, grain counts from individual cells indicated that the mRNA was more abundant on a per cell basis than in the adult CP. After P16, DARPP-32 mRNA levels decreased slightly to adult levels. On both P0 and P7, DARPP-32 mRNA was detected in patches in the CP, similar to that observed for the protein. DARPP-32 mRNA was also detected in the olfactory tubercle and nucleus accumbens on P0, and its development in these two areas proceeded with a similar time course as in the CP. The failure to reliably observe DARPP-32 mRNA prior to birth indicates that the levels are below the sensitivity of the technique.

523.5

DEVELOPMENTAL REGULATION OF HUMAN BRAIN THYMOSIN BETA-10 & ITS MODULATION IN NEUROBLASTOMA CELLS BY RETINOIDS. Alan K. Hall*1, James Hempstead*2, and James I Morgan*2, (Spon:T.W. Lysz).
(1) Section of Urology, Department of Surgery, UMDNJ-Newark, New Jersey 07103-2757,(2)Department of Neurosciences, Roche Institute of Molecular Biology, Nutley,New Jersey 07110.

We used high performance liquid chromatography to identify a peptide which was enriched in rat embryonic and neonatal brain tissue, but absent in the adult CNS. The peptide was found to be thymosin beta-10.(Ziai et al, 1989). Comparison of HPLC chromatograms obtained from adult (90 year old) and fetal (49 days post-conception) human brain tissue indicated that thymosin beta-10 is also subject to developmental regulation in the human CNS. An abrupt loss of neural thymosin beta-10 occurred during the second and third trimesters of pregnancy and levels attained a nadir in the adult brain. We used a number of human tumor cell lines derived from the nervous system to investigate the mechanism which may contribute to the control of thymosin beta-10 gene during neurogenesis. Screening of some 30 cell lines showed that most neuronal cell types expressed thymosin beta-10. The IMR-32 and HTB-10 human neuroblastoma cell lines, in particular, produced thymosin beta-10 as a major peptide species. Retinoic acid induced an inhibition of thymosin beta-10 in HTB-10 cells thus mimicking the in vivo situation. Hence, these observations suggest that the brain is a target for retinoids.

523.6

DIFFERENTIAL EXPRESSION OF CONNEXIN mRNAs DURING NEURAL DEVELOPMENT. D.J. Belliveau and C.C.G. Naus. Dept. of Anatomy, University of Western Ont., London, Canada, N6A 5C1.

The developmental appearance of gap junction mRNAs was examined during postnatal development of the rat and mouse brain. Two cDNAs, one specific for the liver-type gap junction connexin32, and one specific for the heart-type gap junction connexin43, were used to probe Northern blots of total RNA isolated from the forebrain and hindbrain of rats and mice at different pre- and postnatal times. Prior to day 10, connexin32 mRNA is not detectable by Northern blotting. By days 10-15, connexin32 mRNA is evident. This mRNA appears in the hindbrain several days prior to its appearance in the forebrain. The level of connexin32 mRNA increases steadily to day 30, subsequently decreasing by the adult stage. In contrast, connexin43 mRNA is readily detectable as early as embryonic day 18. Postnatally, there is a steady increase in the level of connexin43 mRNA to day 30, followed by a slight decrease in the adult. These data suggest that there is heterogeneity in the gap junctions in brain, and that the mRNAs for two of these gap junction proteins are differentially regulated during neural development.

Supported by MRC, NSERC, Ministry of Community and Social Services Lottery Grant Program, Ontario Mental Health Foundation, and Hospital for Sick Children Fdn.

523.7

cDNA CLONING OF RETINA COGNIN, AN EMBRYONIC CHICK CELL RECOGNITION PROTEIN. A.S.M. Krishna Rao and R.E. Hausman, Department of Biology, Boston University, 2 Cummington St., Boston, MA 02215 USA.

mRNA was prepared from eight day embryonic chick neural retina tissue and used to make cDNA. Expression libraries were constructed in lambda GT vectors. These were screened with the specific polyclonal antiserum against retina cognin [Dobi et al., *Cell Different.* 22, 115-124, 1988] using both alkaline phosphatase-conjugated and ^{125}[I]-labeled secondary antibodies. Multiple screening yielded five clones positive for both these techniques. These clones are currently being investigated for their retina cognin specificity by dot blotting and comparing their temporal patterns of expression during embryonic development with the known pattern of retina cognin expression. The inserts are also being cloned into pbluescript vectors for sequencing and the construction of anti-sense RNA to be used for interference with cognin expression during retina development and with the differentiation of retina neurons *in vitro*. [Supported by NIH grant EY04461 to R.E.H]

523.8

B-50/GAP43 EXPRESSION AND NEURITOGENESIS BEGIN SIMULTANEOUSLY IN THE EMBRYONIC MOUSE NERVOUS SYSTEM S. Biffo*, J. Verhaagen*, L.H. Schrama*, P. Schotman*, W. Danho* and F.L. Margolis (SPON: R. Margolskee) Roche Inst. Molec. Biol. Nutley, N.J. 07110, Rudolf Magnus Inst. for Pharmacology, Utrecht, The Netherlands and Peptide Research Department, Hoffmann-La Roche , Inc., N J.07110

We tested the hypothesis that B-50/GAP43, a membrane phosphoprotein, is involved in neuritogenesis by studying the developmental pattern of expression of B-50/GAP43 mRNA and protein during mouse neuroembryogenesis. B-50/GAP43 mRNA was first detected at embryonic day 8.5 (E8.5) in the presumptive acoustico-facialis ganglion. Subsequently, B-50/GAP43 mRNA and protein were found coexpressed in a series of neural structures: in the ventral neural tube (from E9.5) and dorsal root ganglia (from E10.5), in the marginal layer of the neuroepithelium surrounding the brain vesicles and in the cranial ganglia (from E9.5), in the autonomic nervous system (from E10.5), in the olfactory neuroepithelium and in the mesenteric nervous system (from E11.5), in a continuum of brain regions (from E12.5) and in the retina (from E13.5). B-50/GAP43 immunoreactive fibers were always seen arising from the regions that contained B-50/GAP43 mRNA. The spatial and temporal expression pattern of B-50/GAP43 demonstrates that this protein is absent from neuroblasts and consistently appears in neurons elaborating fibers, independent of their embryological origin. This suggests that B-50/GAP43 can be involved in processes common to all neurons undergoing fiber outgrowth.

523.9

DEVELOPMENTALLY REGULATED EXPRESSION OF PHOSPHOPROTEIN p19 IN RAT BRAIN STUDIED BY IMMUNOCYTOCHEMISTRY. J.A. Amat* U.K. Schubart* and K.L. Fields. Depts. of Neurology and Medicine, A. Einstein Coll. Med., Bronx, N.Y. 10461.

P19, a cytosolic protein that undergoes hormonally regulated phosphorylation im neuroendocrine tumor cells, was previously purified from bovine brain. Its cDNA was recently cloned and sequenced, revealing that the gene encoding p19 has been highly conserved during mammalian evolution and is related to the gene encoding SCG10, a protein expressed in embryonic and perinatal neurons.Using immunoblotting it was previously shown that expression of p19 is restricted in adults to brain and testis. In rat brain it peaks at birth and declines to a plateau of about 5% within 4 weeks. We now present the cellular distribution of p19 immunoreactivity (p19i) in rat brain tissue sections at ages E13, E15, PN1, 12 and 60. The expression of p19i by subsets of neurons and glia is detectable at all ages examined, but it changes dramatically with age in each region. P19i is abundant in many, perhaps all, young neurons during embryonic and perinatal periods, but by 60 days only a restricted set of neurons in cortex, hippocampus, caudal hypothalamus, and brain stem remain strongly positive. Oligodendroglia and some astroctyes in the spinal cord are also positive in adults, but sites of glial proliferation in the subventricular zones are more strongly positive. These data suggest that p19 is a phosphoprotein involved in the differentiation of several cell lineages of the CNS. (Supported by NIH grants NS14580, NS07098, and NS26333).

523.10

REGULATED EXPRESSION OF NERVE GROWTH FACTOR-RECEPTOR MOLECULES IN BRAIN MORPHOGENESIS. E. Escandon* and M. Chao*.(SPON:I. Black). Dept. of Cell Biol. and Anatomy, Cornell University Medical College, New York, NY 10021.

Expression of the NGF receptor gene is tightly regulated during chicken brain development. We describe here the profile of appearance of NGF receptor protein during chicken development. Using ^{125}I-NGF and the crosslinking agent EDC, we identified a major specific crosslinking species of 100,000 MW, identical to the one found in PC12 cells. The highest level of crosslinked receptor was observed in chicken retina cells. In chicken brain, NGF receptor was detected as early as embryonic day 4, and reached a peak of expression at embryonic days 6-8, decreasing with embryonic development to near undetectable levels at birth. The same developmental pattern was observed in retina and the different brain regions (telencephalon, diencephalon, mesencephalon and metencephalon). Receptor binding activity is identical to the pattern of steady state levels of receptor mRNA, suggesting that transcription of the gene can be directly correlated with the appearance of the receptor protein.

523.11

NERVE-TARGET INTERACTIONS IN THE DEVELOPING SYMPATHETIC NERVOUS SYSTEM: DEVELOPMENT OF AN m3 MUSCARINIC CHOLINERGIC RECEPTOR. M. P. Grant and S. C. Landis Dept. of Pharm. and Center for Neurosciences, Case Western Reserve University, Cleveland OH 44106.

Relatively little is known about the developmental mechanisms which regulate receptor expression. Rat sweat glands provide a model to study the relationship between transmitter phenotype and receptor expression because the innervation changes from adrenergic to cholinergic.

To examine the relationship between gland innervation and receptor expression, we characterized the adult receptor, quantitated the development of muscarinic receptors, and determined the concentration of receptors in two experimental paradigms. In adult glands, the receptor demonstrates a pharmacological profile consistent with an M2 (glandular) subtype; it displays high affinity for 4-DAMP, intermediate affinity for pirenzepine, and low affinity for AF DX-116. Preliminary *in situ* hybridization studies suggest that glands express the m3 molecular subtype (Bonner, T. I. TINS 12: 149). During development the forming glands first express muscarinic binding sites on P4. On P14, shortly after cholinergic properties appear in the innervation, but when only a minority of glands respond to nerve stimulation, the concentration of binding sites approaches adult levels. Glands that develop in the absence of innervation or glands from adult animals that are acutely denervated express a reduced concentration of receptors (66% and 80% respectively), of unchanged Kd and are nonresponsive to muscarinic agonist. Our results suggest that a significant proportion of muscarinic ligand binding sites are expressed in the absence of functional cholinergic innervation, but that this innervation is important in the development of cholinergic responsiveness in rat sweat glands.

523.12

EXPRESSION OF THE D_2-DOPAMINE RECEPTOR IN THE DEVELOPING RAT BRAIN. M.M. Durand*, D.C. Chugani*, and M.E. Phelps. (SPON: J.C. Mazziotta) Division of Nuclear Medicine and Biophysics, Department of Radiological Sciences, UCLA, School of Medicine, Los Angeles, CA 90024, USA.

As a first step in our effort to produce a cell line containing the D_2-dopamine receptor, we have investigated the developmental pattern of the mRNA encoding for the D_2-receptor using a cDNA probe (Bunzov J.R. et al., Nature, 336:783, 1988) and the cellular localization of this receptor. During embryogenesis, mRNA was detectable in Northern blot at a low level as early as e12, followed by a subsequent increase in the last stages of embryogenesis. The same developmental pattern can be observed in striatum and mesencephalon. In neuronal cultures from 14-day-old embryonic striatum, the level of mRNA increased significantly during neuronal sprouting (1 to 5 days in culture). The higher level of mRNA was detected at the beginning of synaptogenesis (5 days in culture) and the number of cells mapped by in situ hybridization with specific labelled cDNA reached 40%. Then, during neuronal maturation (5 to 11 days in culture), the level of mRNA plateaued. Using an antibody against the spiperone analog, carboxy-oxime-spiperone, the D_2-receptor appeared at 7 days in culture in small segments of neurites. The colocalization of the D_2-receptor with specific neuronal markers to identify the cell types containing this receptor in striatum and mesencephalon will be presented.

523.13

PROTEASE INHIBITOR ACTIVITY AND GENE EXPRESSION IN THE DEVELOPING ANTENNAE AND BRAIN OF *MANDUCA SEXTA*. E. H. Hanneman, M. R. Kanost*, H. K. Lehman, and J. G. Hildebrand. ARL Div. of Neurobiology and Dept. of Biochemistry, Univ. of Arizona, Tucson, AZ 85721.

Proteases and their inhibitors are expressed at critical stages in the development of individual neurons and in the overall pattern of development. Inhibition of proteases released from neuronal growth cones decreases cell migration and sprouting, and glial cells can release protease inhibitors. Proteases also have global roles in pattern formation, such as the *snake* and *easter* mutations that affect the dorsal-ventral axis in *Drosophila melanogaster*.

The brain of the sphinx moth *Manduca sexta* undergoes extensive remodeling during metamorphosis, a period when certain larval neurons and networks degenerate and new adult structures form. We used a simple assay to show that stage-6 pupal brain contains protease inhibitor activity for both trypsin and chymotrypsin. Northern analysis at low stringency using a serine protease inhibitor (serpin) cDNA isolated from *Manduca* fat body shows that stage-18 brain and stage-6 antennae contain a 1.6 kb transcript that hybridizes to the 1.4 kb serpin cDNA.

The protease inhibitors are currently being purified. The cloning of this transcript and study of its localization will help to determine how proteases and their inhibitors affect the restructuring of the *Manduca* nervous system during metamorphic adult development. [Supported by grants from the NIH, NSF, and American Cancer Society.]

523.14

ADULT MOTONEURON MORPHOLOGY DEPENDS ON FUNCTIONAL CONNECTIONS. S. Vanden Noven, N.L. Wallace* and M.J. Pinter. Dept. of Anatomy, Medical College of Pennsylvania, Philadelphia, PA 19129.

We studied uptake and retrograde transport of intramuscularly-injected horseradish peroxidase (HRP) by axotomized rat medial gastrocnemius (MG) motoneurons, that were allowed to regenerate but prevented from reinnervating muscle. The MG nerve was cut and the proximal end sutured onto the normally-innervated lateral gastrocnemius (LG) muscle. To prevent reinnervation, the denervated MG muscle was removed. At various days post-operative (DPO, 7-200), HRP was injected into the LG muscle after the LG-soleus nerve was ligated and cut. Cell counts and morphometric measurements were obtained from labeled MG neurons. By 30 DPO and for later DPOs, the somas of MG motoneurons lacking a target were significantly smaller than contralateral, normal MG motoneurons. The number of labeled MG motoneurons was similar on the control and experimental sides for these same DPOs. At 7 DPO, the reduction of soma size on the experimental side was less evident and the number of labeled neurons on the experimental side was consistently lower than on the control side. The results indicate that motoneurons deprived of functional contact with muscle for prolonged periods atrophy, but do not die.

Supported by NIH grants NS24000 and NS24707.

523.15

A MONOCLONAL ANTIBODY TO DEVELOPING RAT NERVOUS TISSUES. T.Seki* and Y.Arai*(SPON: A. Matsumoto). Dept. of Anatomy, Juntendo Univ. Sch. of Med., Tokyo 113, Japan.

A monoclonal antibody(MAb) 12E3 was produced against a cell suspension from the embryonic rat forebrain. This antibody stained the embryonic and neonatal nervous tissues, but not the adult tissues. In the cerebral cortex of 14 day embryos, the immunoreactivity was detected in the horizontally oriented cells(the first neurons to mature in cortex) in the marginal zone, whereas the germinal cells in the ventricular zone were almost negative. In 18 day embryos, the neuronal processes or axons in the intermediate zone and the marginal zone were strongly stained, the germinal cells in the ventricular zone being only weakly stained. In the cortical plate and the subventricular zone, the immunoreactivity was also found in the neuronal processes and cell bodies. After birth, the immunoreactivity was gradually reduced in these neural substrates. No reactivity was found in the adult cortex. These results suggest that the molecules detected with MAb 12E3 are transiently expressed in developing nervous tissues.

524.1

CO-CULTURES OF TEMPERATURE SENSITIVE PC 12 CELLS AND RAT STRIATAL NEURONS: LIGHT AND ELECTRON MICROSCOPIC IMMUNOCYTOCHEMISTRY. T.J. Mahalik, D.M. Rausch, L.E. Eiden and T.E. Finger. Dept. of Cellular and Structural Biology, U. of Colorado Medical School, Denver, CO 80262 and Unit on Cellular and Molecular Neurobiology NIMH, Bethesda MD 20892.

PC12 cells were stably transformed with a replication defective recombinant retrovirus containing a temperature sensitive v-src gene. The resultant cells (PCB8-T_1) proliferate at the non-permissive temperature of 40°, but stop dividing and differentiate at 37°C (Rausch and Eiden, Abst. SN 1988). The purpose of the present in vitro study is to characterize contacts between differentiated PCB8 cells and cultured rat striatal neurons.

Striata were dissected from E14 fetal brains and were mechanically disrupted and plated in 60 mm^2 petri dishes (2 x 10^5/plate). After 7-14 days, 1 x 10^4 undifferentiated PCB8 cells were added to the dishes and were grown at 37° for an additional 7-10 days. Cells were fixed and prepared for electron microscopy and immunocytochemistry.

PCB8-T_1 cells extended extensive networks of neurites within 7 days of co-culture. The majority of PCB8-T_1 processes were confined to areas of plates which were free of striatal cells. Nevertheless, light microscopic immunocytochemistry revealed that tyrosine hydroxylase-like immunoreactive processes from PCB8-T_1 cells were in contact with unlabeled striatal neurons. At the EM level, vesicle-filled PCB8-T_1 processes made contacts with non-neuronal and neuronal cells; morphologically differentiated synapses were, however not present. Our results indicate that PCB8-T_1 cells can differentiate and make contact with striatal target cells. We are presently conducting both in vivo and in vitro studies to further characterize the interactions between PCB8-T_1 cells and striatal neurons.

524.2

TISSUE PLASMINOGEN ACTIVATOR BINDS TO NEURONAL THY-1. J. H. Ware* and R. N. Pittman (SPON: G. B. Koelle) Mahoney Institute of Neurological Sceinces and Dept. of Pharmacology, School of Medicine, University of Pennsylvania, Philadelphia, PA 19104

Plasminogen activators (PA) are a class of serine proteases which convert the zymogen plasminogen into the fibrinolytic protease plasmin. PAs have been implicated in many events in the developing nervous system, including proliferation, migration, and neurite outgrowth. Mechanisms underlying the role of PAs in these events are unknown. We are currently investigating the possibility that cell surface binding sites for PA are involved in these events. The present experiments were carried out to identify the neuronal binding site of tissue-type plasminogen activator (tPA). Crosslinking ^{125}I-tPA to PC12 cell surfaces indicated a single tPA binding site with a M_r of 30 kDa. This and other preliminary data suggested that the binding site might be the neuronal glycoprotein Thy-1. Binding of ^{125}I-tPA to rat sympathetic neurons and PC12 cells was inhibited by monoclonal antibodies specific for Thy-1, but not by antibodies specific for other cell surface glycoproteins. The ^{125}I-tPA binding properties of purified rat brain Thy-1 were then compared to those of PC12 cells. ^{125}I-tPA bound to Thy-1 with a $k_1 = 2.8x10^5$ min^{-1} M^{-1}, and to PC12 cells with a $k_1 = 4.3x10^5$ min^{-1} M^{-1}. The k_{-1} values were $7.1x10^{-3}$ min^{-1} for Thy-1 and $6.2x10^{-3}$ min^{-1} for PC12 cells. Kinetically determined K_d values were 25 nM for Thy-1 and 14 nM for PC12 cells. Scatchard analysis of saturation isotherms yielded K_d values of 15±9 nM for Thy-1 and 23±10 nM for PC12 cells. The proteolytic site of tPA does not appear to be involved in binding to Thy-1. The involvement of other domains of tPA is being investigated, as is the functional significance of this interaction. Supported by the McKnight Foundation and NIH #NS22663.

524.3

MORPHOLOGICAL ALTERATION AND INHIBITION OF NGF INDUCED NEURITE OUTGROWTH IN PC12 CELLS OVEREXPRESSING C-MYB OR ORNITHINE DECARBOXYLASE **S. C. Feinstein, L. G. Marschall* and G. Vescovo* Neuroscience Research Institute and Dept of Biological Sciences, University of California, Santa Barbara, CA 93106**

We have examined the effects of two gene activities, ornithine decarboxylase (ODC) and c-myb, on PC12 cells and their ability to respond to NGF. We have overexpressed ODC in PC12 cells by two independent strategies. Cells have been grown in increasing concentrations of difluormethylornithine, a suicide substrate inhibitor of ODC, and clones vastly overexpressing ODC isolated. Secondly, DNA molecules containing a full length rat ODC cDNA have been stably transfected into cells and clones isolated. In both cases, cells are dramatically altered. They are extremely flattened with very prominant nuclei and nucleoli. Additionally, ODC transfectants fail to project neurites in response to NGF. Transfection of the same DNA molecule but with the ODC cDNA inverted relative to the promoter yields cells that do project neurites when treated with NGF.

We have also stably introduced the c-myb gene into PC12 cells. Clonal isolates display a similar phenotype as the ODC overexpressing cells, i.e., flat morphology and failure to project neurites in response to NGF.

This study identifies two genes whose excess activity alters cell morphology and responsiveness to NGF in a similar manner. It is possible that each influences a common biochemical pathway. Further investigation of these effects may lead to a better understanding of the mechanisms of NGF action and neuronal differentiation. (Supported by the Muscular Dystrophy Association and NIH Grant #RO1-NS24387)

524.4

CONE PHOTORECEPTOR-SPECIFIC MOLECULES EXPRESSED BY NEURONALLY DIFFERENTIATING Y79 HUMAN RETINOBLASTOMA CELLS. G. Massry*, J. Tombran-Tink* and L.V. Johnson (SPON: M. Cullen). Department of Anatomy & Cell Biology, USC School of Medicine, Los Angeles, CA.

Y79 human retinoblastoma cells can be induced to differentiate along neuronal pathways by medium conditioned by cultures of human fetal retinal pigmented epithelial cells (RPE-CM) (Tombran-Tink and Johnson, Invest. Ophthalmol. Vis. Sci. 29:243, 1988). We have examined the expression of several cone photoreceptor markers during RPE-CM-induced neurogenesis of Y79 cells. The probes employed were cone-specific monoclonal antibodies 1 and 2 (CSA-1, CSA-2) and peanut lectin (PNA). CSA-1, 2 and PNA label distinct and separate regions of cone photoreceptor cells in a variety of vertebrate retinae.

In Y79 cells induced to undergo neuronal differentiation, these markers show marked temporal and spatial differences in expression. CSA-1, CSA-2 and PNA bind only weakly to undifferentiated Y79 cells, but show increased labeling of differentiated cells, each at different intervals post-stimulation with RPE-CM. CSA-1 binding appears polarized and cytoplasmic, while the CSA-2 binding pattern appears more punctate. PNA shows a gradient of binding decreasing in intensity from cell body to axon.

These findings indicate that neuronal differentiation of retinoblastoma cells involves the expression of several molecular species characteristic of cone photoreceptor cells in the retina. (Supported by NIH EY04741.)

524.5

MODULATION OF CATECHOLAMINES IN RETINOBLASTOMA CELLS INDUCED TO DIFFERENTIATE BY RETINAL PIGMENTED EPITHELIAL CELL CONDITIONED MEDIUM. J. Tombran-Tink*[1], L.V. Johnson[1], J. Adams*[2] and L. Klaidman*[2]. [1]Dept. of Anatomy & Cell Biology and [2]School of Pharmacy, USC School of Medicine, Los Angeles, CA.

Previously we described the induction of neuronal differentiation of human Y79 retinoblastoma cells by medium conditioned by human fetal retinal pigmented epithelial cells (RPE-CM). We have examined modulation of catecholamine content in undifferentiated, RPE-CM stimulated unattached, and 10 days attached differentiated Y79 retinoblastoma cells by HPLC. These analyses reveal decreased dopamine levels and elevated epinephrine content in differentiated Y79 cells. Dopamine is present at 25 picomoles/mg in non-differentiated Y79 cells while epinephrine levels are undetectable. In contrast, differentiated Y79 cells show undetectable levels of dopamine but increased epinephrine levels to 297.8 pm/mg and trace amounts of putative norepinephrine. It was further observed that epinephrine levels are elevated by 4-8 days exposure to RPE-CM, prior to attachment and differentiation. Such "stimulated" cells contain 140.6 pm/mg epinephrine and low levels of putative norepinephine while dopamine content is already decreased to undetectable levels. These studies suggest that enzymes such as dopamine B-hydroxylase and norepinephrine N-methyltrasferase may be activated during the differentiative process. (Supported by NIH EY04741).

524.6

NEURONAL DIFFERENTIATION OF P19 CELLS: CORRELATION BETWEEN MORPHOLOGY AND MEMBRANE CURRENTS. L.E. Cheun and H.H. Yeh. Dept. Neurobiology and Anatomy, U. Rochester Sch. Med., Rochester, NY 14642.

The P19 embryonal carcinoma cell line was used as a model for examining the development of ionic currents in the differentiation of pluripotent cells toward a neuronal phenotype. Retinoic acid (RA; 0.1 uM) was used as the differentiating agent (Jones-Villeneuve et al., J. Cell Biol. 94, 1982). We examined whole-cell current profiles of P19 cells before, during, and after differentiation using the patch-clamp technique. An attempt was made to reveal the morphology and to establish the presence of neuron-specific enolase (NSE)-like immunoreactivity in the cells studied, so that expression of ionic currents could be correlated with a known neuronal marker.

Undifferentiated P19 cells either expressed no currents or expressed a voltage-dependent, TEA-sensitive outward current. No correlation was found between the expression of currents and that of NSE-like immunoreactivity at this undifferentiated state. Cells that were in the initial stages of RA treatment resembled the undifferentiated cells in terms of their morphology, electrical properties and NSE-like immunoreactivity. In contrast, cells which had undergone the full differentiation protocol expressed, in addition to the previously observed outward current, a large voltage-dependent inward current. Invariably, such cells emitted neurites and were NSE-like immunoreactive. The predominant component of the inward current was a TTX-sensitive sodium current. A small, cadmium-sensitive calcium current could also be revealed. Inward currents of low amplitude could first be detected by 18 hours after the completion of the RA-treatment. The amplitude increased progressively over the subsequent 3 days in culture and tended to decrease thereafter.

The morphological and histochemical features of differentiated P19 cells, previously reported by others, were extended here to include excitable membrane properties. Thus, differentiated P19 cells express a spectrum of features characteristic of neurons. Our correlative study indicates that the full complement of morphological, histochemical as well as excitable membrane properties need to become manifest before a cell committed toward a neuronal phenotype can be considered to be fully differentiated.

Supported by PHS grants NS 24830 and NS 01340.

524.7

NEURONAL ULTRASTRUCTURAL CHARACTERISTICS OF A CHOLINERGIC CELL LINE. D.N. Hammond, H.J. Lee, and B.H. Wainer. Departments of Pediatrics and Neurology and the Committee on Neurobiology, The University of Chicago, Chicago, IL 60637.

A hybrid cell line expressing differentiated characteristics typical of cholinergic neurons has been derived from the murine septal region. In addition to choline acetyltransferase and acetylcholinesterase activity and the expression of neurofilament protein, SN17 cells display neuronal morphological characteristics at the light microscopic level. We have thus undertaken a detailed study to determine the differentiated ultrastructural traits expressed by the line. Cells are grown in Dulbecco's modification of Eagle's medium containing 10% fetal calf serum. They are fixed with 1% paraformaldehyde and 2% glutaradlehyde, postfixed with osmium tetroxide, ultrathin sectioned, stained with uranyl acctate and lead citrate, and examined under a Philips 201 electron microscope. The somata contained numerous mitochondria and prominent rough endoplasmic reticulum. Membrane specializations, with maintenance of the intercellular cleft but no adjacent vesicles, are also present. The neurites display prominent varicosities containing, in addition to intermediate filaments and microtubules, clear and dense core vesicle, and membrane specializations. The distal ends of the neurites are typical neuronal growth cones, displaying lamellipodia, a complex meshwork of microfilaments, and numerous mitochondria, polyribosomes, and vesicles. SN17 cells thus express, under routine culture conditions and in the absence of any differentiating agents, a number of ultrastructural characteristics typical of neurons. (Supported by NIH grants NS 01244, NS 25787, and T32HD070009, and grants from the Illinois Department of Public Health and the Alzheimer Disease and Related Disorders Association.)

524.8

NEUROTRANSMITTER CONTENT AND MONOAMINE OXIDASE ACTIVITY IN THE ADRENERGIC/SEROTONERGIC NEUROBLASTOMA CELL LINE LA-N-1. S. Sullivan, S.Y. Felten, and M.F.D. Notter. Dept. Neurobiology-Anatomy, University of Rochester Medical Center, Rochester, New York 14642.

The human neuroblastoma cell line LA-N-1 has previously been characterized as adrenergic based on tyrosine hydroxylase (TH) activity and immunocytochemical staining for TH, dopamine beta hydroxylase (DBH) and phenylethanolamine-N-methyl transferase (PNMT). Although enzyme assays showed no activity for DBH or PNMT, we demonstrated that staining for TH and DBH increased after induced cellular differentiation. To explore this discrepancy between methods, we used a highly sensitive HPLC assay to test for the storage of catecholamine products. We found that these cells do not contain dopamine, but do contain norepinephrine (NE), epinephrine (E) and a significant amount of serotonin (5HT). The amount of neurotransmitter product stored is dependent upon the differentiation state of the cells. Mitotic cells contain 17.8 pMol NE/mg protein, 344.5 pMol E/mg protein and 52.4 pMol 5HT/mg protein. After differentiation for 4 days with 10^{-5} M retinoic acid, the cells contain 68.1 pMol NE/mg protein, 261.3 pMol E/mg protein and 10.79 pMol 5HT/mg protein.

Since we have previously shown that LA-N-1 cells are sensitive to 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) in a dose dependent manner and many clonal lines contain monoamine oxidase (MAO), we explored the possibility that these cells possess MAO activity. Initial studies using a fluorescent method utilizing kynuramine as substrate show MAO activity in both mitotic and chemically differentiated cells. Preliminary results indicate that these cells might contain both MAO type A and B, although this remains to be fully substantiated.

(Supported by NIH NS 25778)

524.9

IMMUNOCYTOCHEMICAL AND HISOTCHEMICAL CHARACTERIZATION OF ORGANOTYPIC SLICE CULTURES OF CEREBRAL CORTEX GROWN IN DEFINED MEDIUM. C.M. Annis, R.T. Robertson and J. Yu. Departments of Anatomy and Neurobiology and Physical Medicine and Rehabilitation, University of California, Irvine, CA 92717.

We are studying the development of cerebral cortex and certain cortical afferents, using the organotypic tissue slice culture method of Gahwiler (TINS, 1988). We report here the characterization of cortical neurons that survive in culture, with regard to immunocytochemical and histochemical staining properties. Slices of parieto-temporal cortex were obtained from rat pups aged 3-5 days. Slices were cultured on collagen coated coverslips in defined medium (Annis et al., 1989) for 1-4 weeks. Following fixation, cultures were processed for acetylcholinesterase (AChE), cytochrome oxidase (CO), choline acetyl transferase (ChAT), gamma-aminobutyric acid (GABA), glutamate, glial fibrillary acidic protein (GFAP) or one of several neuropeptides.

Cultures of cortex grow well in defined medium and maintain normal gross cortical appearance. Histochemical and immunocytochemical studies demonstrate that a variety of types of neurons survive in culture. Neurons stain for AChE or ChAT, but AChE-positive neurons are found more frequently in culture than *in vivo*. Multipolar neurons stained for GABA, whereas neurons with pyramidal morphologies stained for glutamate. These results indicate that cortical slice cultures maintain many of the same peptides and neurotransmitters that are found *in vivo*, and provide a promising model for the study of cortical development.

Supported by NSF grant 87-08515 and NIH grant NS 25674.

SOMATOSENSORY SYSTEM II

525.1

ORGANIZATION OF THE PERIPHERAL PROJECTIONS OF THE TRIGEMINAL GANGLION IN FETAL RATS. G.J. Macdonald, N.L. Chiaia and Robert W. Rhoades (SPON: T.H. Chiu). Depts. of Anatomy, Medical College of Ohio, Toledo, OH 43699 and Robert Wood Johnson Medical School, Piscataway, NJ 08854.

Retrograde tracing with true blue (TB) and diamidino yellow (DY) was used to evaluate the topography of the peripheral projections of the trigeminal (V) ganglion in rats on embryonic day 16 (E-16). Timed pregnant rats were anesthetized, E-16 fetuses were exposed, and small injections of TB and DY were made into the face. After 8-12 hrs, fetuses were harvested and tissue was prepared for fluorescence microscopy. At E-16, peripheral V ganglionic projections were quite adult-like. Cells that projected to the vibrissa pad were restricted to the ophthalmic-maxillary part of the ganglion with those innervating dorsal (A- and B-) row follicles located medially and those supplying ventral (D- and E-) rows laterally. Ganglion cells that innervated the middle (C-) row of vibrissa follicles were located in center of the mediolateral extent of the ophthalmic-maxillary region. Injections into the ophthalmic skin or the cornea labelled cells that were clustered in the most dorsal and anteromedial portions of the ophthalmic-maxillary region. Tracer injections into the lower jaw or the skin just rostral to the ear labelled cells that were restricted to the lateral and ventral part of the ganglion. None of the combinations of injections that we carried out produced large numbers of double labelled cells. Small numbers (<15 per ganglion) of double labelled cells were seen after injection of TB into the vibrissa pad and DY into the upper lip. This was also the case after paired injections into the lower lip and jaw, respectively. All of these results indicate that the peripheral projections of the V ganglion are quite adult-like from the earliest age at which we could detect them with retrograde tracing techniques.

Supported by DE 07734, BNS 85 17537, and funds from the State of Ohio Research Challenge.

525.2

DEVELOPMENT OF INPUT PATTERNS FROM THE DIGITS TO THE SPINAL CORD AND CUNEATE NUCLEUS IN MONKEYS. Florence, S.L. and J.H. Kaas. Dept. Psychology, Vanderbilt Univ., Nashville, TN 37240. Injections of transganglionically transported HRP conjugates into the digit tips of a 5 day and 1 month old macaque monkey (Macaca fascicularis) demonstrated exuberant terminations of peripheral nerve afferents in the dorsal horn of the spinal cord but adult-like restrictions of terminations in the cuneate nucleus of the brainstem. In the spinal cord of the neonate, terminations extend rostrocaudally across almost two thirds a cervical segment in the superficial dorsal horn and span 2 segments deeper in the dorsal horn. The deeper label is less dense and discontinuous, forming a sequence of small (100um) patches of label separated by unlabeled gaps of comparable size. A similar pattern was found in the dorsal horn of the 1 month old monkey. In adults (Florence, et al., 1989), terminations are found only in the superficial dorsal horn and are restricted to less than half a segment. In the pars rotunda of the cuneate nucleus, digit injections result in discrete foci of label restricted within specific cytochrome oxidase dense clusters in both the neonate and adult monkeys. Rostrally and caudally, the label is less dense and more widespread. Thus, the pattern of inputs to the dorsal horn, even one month after birth, is not as discrete as in adults. Yet by birth, projections to the cuneate nucleus are adult-like. (Supported by NS16446 and NS08062).

525.3

ROLE OF POSTNATAL PRIMARY AFFERENT ACTIVITY IN CENTRAL TRIGEMINAL PATTERN FORMATION. T.A. Henderson, T.A. Woolsey & M.F. Jacquin. Dept. of Anat. & Neurobiol., St. Louis Univ. Sch. Med., St. Louis, MO 63104 & Div. of Exp. Neurol. & Neurosurg. & McDonnell Center for Studies of Higher Brain Function, Washington Univ. Sch. Med., St. Louis, MO 63110.

Central trigeminal (V) nuclei receive initially diffuse, though topographic, inputs early in development. Mechanisms dictating V afferent segregation into barrel-like patches have not been elucidated, though lesion studies implicate a peripheral factor(s). One candidate is patterned neuronal activity. To test this hypothesis, a slow release polymer (ELVAX40; Reh & Constantine-Paton, J. Neurosci. 5, '85) containing .001M TTX was placed under the left infraorbital nerve in newborn rats. Recordings in perinates showed V ganglion cells were silenced and unresponsive to peripheral stimuli, yet discharged by brainstem shocks. HRP transport channels were intact and ganglion cell #'s appeared normal. On postnatal day 5-adulthood (N=7), cytochrome oxidase (CO) staining patterns in the V brainstem complex, VPM and barrel cortex were indistinct from normal. Patch measurements also did not differ from normal (ANOVA, p=.87). This negative result was corroborated in 7 rats subjected to another form of deprivation. From birth to sacrifice at days 5-9, whiskers were trimmed daily and 0.5% bupyvacaine was injected into the left whisker pad at 2.5-4 hr intervals. CO patches again did not differ from normal (p=.84). Thus, V pattern formation occurs even when the entire infraorbital nerve is silenced from birth. NIH DE07734, DE07662, NS17663

525.4

NEONATAL INFRAORBITAL NERVE SECTION: DIFFERENTIAL EFFECTS ON TRIGEMINAL BRAINSTEM CELL NUMBER, SIZE AND DISTRIBUTION IN RAT. T.A. Choy*, T.A. Henderson & M.F. Jacquin (SPON: L.C. Massopust). Dept. of Anatomy & Neurobiology, St. Louis University School of Medicine, St. Louis, MO 63104.

We (Soc. Neurosci. Abstr. 14:1166, '88) have previously shown that infraorbital nerve cut at birth produces a 21% reduction in HRP-labeled projection cells in the trigeminal brainstem complex. Different morphometric methods were used to replicate this finding and to estimate effects on local circuit cell number and distribution. Nissl-stained nucleoli in 10 um paraffin sections were reduced in number by 31.4, 35.2, and 44.6% in principalis, oralis and interpolaris, respectively. Such differential cell counts in spinal vs principal trigeminal nuclei and in Nissl- vs HRP-labeled projection neurons suggest that local circuit neurons die in higher %'s than projection neurons. Yet, numbers of Nissl-stained laminae I-II cells in caudalis were normal. Preservation of these local circuit cells is likely due to the selective sparing of infraorbital inputs to laminae I-II (Br. Res. 269:135, '83).

Differential effects on cell area and density were also found. Thalamic-projecting cells were reliably larger than normal in principalis (9%), but smaller in oralis (11%) and unaffected in interpolaris and caudalis. Reliable reductions of 5-12% also occurred for cerebellar, collicular, and spinal projecting cells, but only in some subnuclei. Similarly, projection cell density changed only in some projection groups and subnuclei. Support: NIH DE07734, DE07662.

525.5

NEONATAL INFRAORBITAL NERVE SECTION: EFFECTS ON CAUDALIS CELL STRUCTURE-FUNCTION RELATIONSHIPS. M. Barcia* & M.F. Jacquin (SPON: K.L. Rossner). Dept. of Anat. & Neurobiol., St. Louis University School of Medicine, MO 63104.

Prior studies indicate that caudalis gelatinous and magnocellular regions respond differently to infraorbital nerve section at birth in rat. Normal cell numbers and afferent inputs are maintained in laminae I and II, but not in III-V. To assess how these differences impact upon single cells, intracellular recording, receptive field mapping, and HRP injection techniques were applied to 11 adult caudalis local circuit neurons ipsilateral to the lesion. Data were compared to 31 normal cells. Deafferented II cells responded only to noxious stimuli applied to maxillary skin or the region of the nerve section. Their morphology did not differ from normal; namely, spiny dendrites restricted to I-III and axon collaterals that arborized in III-V. Deafferented I and III cells also had small receptive fields of a character appropriate to these laminae. Their structure was also within ranges of normal variability. IV cells, however, had large numbers of spiny dendrites with extensive local axon collaterals in II-V. These properties were not seen in normals or in neighboring cells with non-infraorbital receptive fields.

Thus, nerve section at birth did not alter the structure and function of cells in caudalis gelatinous areas, whereas deeper laminae cells were altered. This finding is consistent with afferent input patterns and cell numbers in deafferented caudalis. Support: NIH DE07734, DE07662.

525.6

NEONATAL INFRAORBITAL NERVE SECTION: EFFECTS ON INTERPOLARIS CELL STRUCTURE-FUNCTION RELATIONSHIPS. M.F. Jacquin & T.A. Henderson. Dept. of Anatomy & Neurobiology, St. Louis University School of Medicine, St. Louis, MO 63104.

Deafferentation at birth produces changes in topography, inputs, projection status, cell number, and responses of interpolaris neurons in rat. To determine whether receptive field changes reflect altered somadendritic development, intracellular recording, receptive field mapping, and HRP injection techniques were applied to 43 adult interpolaris cells ipsilateral to the lesion and 54 normal cells.

Number of Proximal Dendrites	NORMAL	DEAFFERENTED
Thalamic-project (mean ± SD)	5.1 ± 1.8	6.5 ± 0.9
Cerebellar-project	5.0 ± 1.2	6.1 ± 1.6
Local Circuit: Vibrissae	4.1 ± 1.2	4.5 ± 1.0
Local Circuit: Non-vibrissae	4.7 ± 1.8	5.6 ± 1.1
Dendritic Tree Transverse Area (μm^2)		
Thalamic-project	82670 ± 46930	66810 ± 38552
Cerebellar-project	48210 ± 31760	45001 ± 17844
Local Circuit: Vibrissae	10860 ± 10870	21617 ± 16595
Local Circuit: Non-vibrissae	73061 ± 30460	64182 ± 27145
Dendritic Tree Transverse Form Factor		
Thalamic-project	0.56 ± 0.20	0.45 ± 0.17
Cerebellar-project	0.70 ± 0.16	0.53 ± 0.19
Local Circuit: Vibrissae	0.61 ± 0.15	0.42 ± 0.11
Local Circuit: Non-vibrissae	0.40 ± 0.15	0.33 ± 0.14

Thus, deafferented cells tended to have more proximal dendrites and radially oriented, oblong dendritic trees with normal transverse areas. Support: NIH DE07734, DE07662

525.7

NEONATAL INFRAORBITAL NERVE SECTION: ULTRASTRUCTURAL COMPARISONS OF NORMAL AND DEAFFERENTED NUCLEUS PRINCIPALIS IN THE RAT. J. Golden, D.S. Zahm & M.F. Jacquin. Dept. Anat. & Neurobiol., St. Louis Univ. Sch. Med., St. Louis, MO 63104.

Infraorbital injury at birth reduces primary afferent inputs to ventral principalis (PrV); spared primary afferents do not sprout into this region. Thus, one prediction is that terminal # in PrV is reduced. To test this hypothesis, EM morphometric methods were applied to maxillary regions of adult PrV ipsi- and contralateral to the lesion. Areas sampled (782 um^2) were = on the 2 sides. We found that terminal and synaptic density increased, and terminal size decreased, in deafferented PrV. In 1 rat, 237 terminals and 86 synapses were encountered vs 158 and 49 on the intact side. Yet, if lesion-induced PrV shrinkage (35%) is considered, estimates of total left and right side terminal and synaptic #'s would be equivalent. There were no changes in the relative %'s of terminals with round (54 vs 54%), flattened (38 vs 37%) or dense-core (6 vs 7%) vesicles, or the relative %'s of synapses on these different terminal types. Terminal areas were reliably smaller than normal (medians: .19 vs .25 um^2; Mann Whitney U test: $p<.01$), reflecting a decreased size of round vesicle terminals only. Similar results were obtained for all of the above in a second rat. In both rats, degenerating terminals were rare and most synapses were asymmetrical and axodendritic. It appeared that deafferented PrV contained a higher % of bundled unmyelinated axons. Afferent sources to PrV remain to be identified. Support: NIH DE07734, DE07662, NS23805.

525.8

DENDRITIC REGRESSION IN DEVELOPING RAT TRIGEMINAL NUCLEUS PRINCIPALIS. N. Hobart*, M.W. Miller & M.F. Jacquin (SPON: N. Connors). Dept. of Anatomy & Neurobiology, St. Louis Univ. Sch. Med., St. Louis, MO 63104 & Dept. of Anatomy, UMDNJ- School of Osteopathic Med., Piscataway, NJ 08854.

Principalis cells exhibit orderly structure-function correlations and topography, require peripheral inputs for normal development, and establish patterns in higher-order trigeminal structures. As a first step in assessing mechanisms underlying their development, somadendritic morphology was studied in adulthood and at 3 postnatal days (PND) when naturally occurring cell death and primary afferent segmentation take place in principalis (birth-PND0, PND2, PND4). Brainstems from 4 animals of each age were processed using standard Golgi-celloidin methods. 40-50 cells of each age were drawn and somadendritic structure quantified. Adult cells typically had polarized short dendrites spanning no greater than a hemisphere around the soma. 5.2 ± 0.3 (mean ± SE) proximal dendrites gave rise to 11.2 ± 1.9 appendages (spines and/or swellings). PND0 cells had radially distributed dendrites that sometimes extended long transverse distances. They had more proximal dendrites (9.3 ± 1.0) than adult cells, but similar #'s of dendritic appendages (12.2 ± 2.0). Perisomatic spines were common and numerous at birth and at PND4. By PND4, most dendritic trees were polarized and this reflected pruning of proximal dendrites. Numbers of proximal dendrites (5.8 ± 0.3) were adultlike, yet appendages were more frequent than in adulthood or PND0 (33.2 ± 8.8). Support: NIH DE07734, DE07662.

525.9

NEONATAL INFRAORBITAL NERVE SECTION: EFFECTS ON TRIGEMINAL SECOND-ORDER COLLATERAL PATTERNS ASSESSED WITH MULTIPLE RETROGRADE TRACERS. P.A. Young, T.A. Henderson, N.L. Chiaia, R.W. Rhoades & M.F. Jacquin. Dept. Anat. & Neurobiol., St. Louis Univ. Sch. Med., St. Louis, MO 63104 & Dept. Anat., Med. Coll. of Ohio, Toledo, OH 43699.

In development, axon collateral patterns are shaped by a number of factors. To assess the role of primary afferent inputs in the development of trigeminal (V) second-order axon branching, the left infraorbital nerve was sectioned at birth and tracers were injected into targets in adulthood. In 6 rats, true blue, diamidino yellow, and HRP were injected bilaterally into thalamus (VB), superior colliculus (SC), and cerebellum (CB), respectively. Labeling patterns were quantified for 11031 cells in V subnucleus interpolaris. On the control right side, 1.5% of the VB-labeled cells also projected to CB and 5.4% of the VB-labeled cells also projected to SC. On the left side, values were 5.3 and 10.2%, respectively. This represented a 249% increase in VB-CB cells and a 91% increase in VB-SC cells. On the control side, 1.0% of the CB-labeled cells also projected to VB. On the deafferented side, this value was 5.5% (449% increase in CB-VB cells). SC-CB double-labeling was never seen on the intact side and in only 2 deafferented cells. Thus, deafferentation increases the relative % of V brainstem cells projecting to more than one target. This could reflect the preservation of a transient collateral(s) that exists at the time of the lesion, or selective survival of multiple-projecting cells. Support: NIH DE07734, DE07662.

525.10

THE DISTRIBUTION OF SPINAL AFFERENTS IN THE THALAMUS AND HYPOTHALAMUS OF NEONATAL RATS. S. Saporta. Dept. of Anatomy, Coll. Med., Univ. South Florida, Tampa, FL 33612.

The normal distribution of spinal afferents to the thalamus was examined in 3 and 5 day old rats using the anterograde transport of horseradish peroxidase conjugated wheat germ agglutinin dissolved in radiocontrast medium injected into the cervical spinal cord 48-72 hrs earlier. Reaction product from anterograde transport was present, in animals injected on postnatal day (PND) 3, in the posterior group; parafascicular, central medial and central lateral nuclei, n. submedius and the zona incerta. A wide extent of the ventral posterior lateral n. (VPL) was labeled with anterograde reaction product. Anterograde Reaction product was also visible in the hypothalamus in the paraventricular n., the lateral and posterior hypothalamic areas. A few axons were visible passing through the periventricular area. Labeled somata were present in the paraventricular n., the lateral hypothalamus and in zona incerta. A similar pattern of label was present on PND 5, though there appeared to be some refinement of the labeling pattern.

525.11

NEUROGENESIS OF SPINOCEREBELLAR NEURONS IN THE LUMBAR SPINAL CORD OF THE RAT. J.A. Beal, K.N. Nandi*, and D.S. Knight*. Department of Cellular Biology and Anatomy, Louisiana State Univ. Med. Ctr., Shreveport, LA 71130.

Studies in our laboratory show that ascending tract (relay) neurons of the rat spinal cord proliferate from embryonic (E) day E12 through E15. Relay neurons which proliferate on day E15 represent only a small percentage of the total population of ascending tract neurons and were observed in spinal cord laminae which give rise to major cerebellar projections. The objective of the present study, then, was to determine if the late dividing relay neurons on day E15 are spinocerebellar neurons. In order to label these cells during their proliferative period, tritiated thymidine was administered to fetal rats on day E15. Spinocerebellar neurons were later labeled in each animal via the retrograde transport of Fluoro-Gold, which was injected unilaterally into the cerebellar hemisphere. Cell counts showed that 25% of the spinocerebellar neurons in the upper lumbar spinal cord were labeled with tritiated thymidine on day E15. This is in contrast to 1 to 4% of the total population of ascending tract neurons labeled with tritiated thymidine at this time. In conclusion, spinocerebellar neurons represent a large proportion, if not all, of the relay neurons dividing on the final day of spinal neurogenesis for ascending tract neurons.

525.12

NEUROGENESIS OF ASCENDING TRACT NEURONS IN THE RAT SPINAL CORD. K.N. Nandi*, J.A. Beal and D.S. Knight* (SPON: J.E. Penny). Department of Cellular Biology and Anatomy, Louisiana State University Medical Center, Shreveport, LA 71130.

The purpose of this study was to determine the neurogenic period for projection neurons of the ascending tracts. In order to label proliferating neurons, tritiated thymidine was administered to fetal rats on embryonic (E) days E12 through E16. Ascending tract neurons of the lumbar cord were later (postnatal days 40-50) labeled in each animal with Fluoro-Gold applied at the site of a hemisection at spinal cord segment C3. Ascending tract neurons which were undergoing mitosis in the upper lumbar cord were double labeled, i.e., labeled with both tritiated thymidine and Fluoro-Gold. Double labeled neurons were observed from days E12 through E15. The maximum number of double labeled neurons, 89% to 92%, was observed on day E13. The number diminishes to 1 to 4% on day E15. Double labeled neurons on day E15 were confined to laminae III, IV, V, and X, and the Nucleus dorsalis and most had ipsilateral projections. Results show that ascending tract neurons continue to proliferate throughout most of the neurogenic period and that most of the neurons undergoing mitosis on the final day of proliferation project ipsilaterally.

DEVELOPMENTAL DISORDERS: HUMAN DISEASES

526.1

LUTEINIZING HORMONE-RELEASING HORMONE (LHRH) CELLS DO NOT MIGRATE NORMALLY IN KALLMANN'S SYNDROME (HYPOGONADOTROPIC HYPOGONADISM WITH ANOSMIA). M.Schwanzel-Fukuda, D.Bick,[1]* and D.W.Pfaff. Rockefeller University, New York,N.Y.and [1] Div.of Genetics and Birth Defects,University of Texas,San Antonio,Texas.

Kallmann's syndrome is a genetic disorder characterized by gonadal dysplasia with decreased secondary sex characteristics, a deficiency of LHRH, and anosmia. The principal endocrine defect is a failure of secretion of LHRH resulting in underdevelopment of the pituitary gonadotropes and an inability to synthesize and release LH and FSH. Examination of serial sections of Bouin's-fixed, paraffin-embedded brain and nasal regions of a Kallmann's fetus, with antiserum to LHRH, showed 1) a striking absence of LHRH cells and fibers in the brain and 2) thick clusters of LHRH cells and fibers in the nose, in branches of the terminalis nerve and in the meninges on the dorsal surface of the cribriform plate. Normal fetal brains, matched for age and sex, had LHRH cells and fibers in the hypothalamus, median eminence, preoptic area and terminalis nerve. Since LHRH cells recently were discovered to migrate from the olfactory placode into the brain (Schwanzel-Fukuda and Pfaff Nature 338:161-164,1989) we can account for the hypogonadotropism of Kallmann's syndrome by a failure of LHRH cells to migrate into the brain. Supported by NIH grant 19962 and funds from the Whitehall Foundation.

526.2

TOURETTE SYNDROME: A NEUROCHEMICAL ANALYSIS OF CORTICAL BRAIN REGIONS. H.S. Singer, I.H. Hahn, E. Nelson, T. Moran, Johns Hopkins University School of Medicine, Baltimore, MD, 21205.

To increase our understanding of the pathophysiology in Tourette syndrome (TS), autopsy samples were assayed for markers of synaptic neurotransmission. Postmortem brain specimens from 4 adults with TS, 3 males & 1 female, ages 38-83 years, were available from Brodmann areas A4, A17 and A38. No significant differences were detected between TS and control specimens for ChAT (mean control = 8.7 $\pm$ 0.4; TS = 8.2 $\pm$ 1.3 nmol/mg prot/hr) or GAD (mean control = 2.4 $\pm$ 0.5; TS = 2.4 $\pm$ 0.6 nmol/mg prot/hr). Similarly, no consistent alterations were identified for the concentration of dopamine, norepinephrine, or the serotonin metabolite 5-HIAA. Muscarinic receptors, measured with [^{3}H]QNB, in all cortical regions were comparable (mean control = 201.4 $\pm$ 15.0; TS = 210.3 $\pm$ 15.3 fmol/mg prot). Beta receptors, measured with [^{125}I]ICYP, were alike in areas A4 and A17 though they were significantly reduced in TS patients in A38.

The primary neurotransmitter, or receptor, abnormality in TS remains controversial. This study has failed to identify a distinct biochemical defect in cortical specimens.

526.3

DOPAMINE-BETA-HYDROXYLASE DEFICIENCY: RESTRICTION ANALYSIS OF LYMPHOCYTIC DNA. S.E. Perry*, A. Lamouroux*, J.A. Phillips, III*, J. Mallet*, I. Biaggioni*, D. Robertson. Clinical Research Center, Vanderbilt University, Nashville, TN 37232.

Dopamine-beta-hydroxylase (DBH) deficiency is a newly-recognized disorder in which patients fail to express sufficient quantities of the functional enzyme. Consequently, they have little or no norepinephrine and epinephrine in the central or peripheral nervous system. In contrast, dopamine, the substrate for DBH, is present in five to tenfold excess. Metabolites of the catecholamines show the same trends as the primary amines. The physical characteristics of the patients include severe orthostatic hypotension, nasal stuffiness, ptosis of the eyelids, hyperextensible joints, and retrograde ejaculation. Patients appear normal in development and in intellectual function. Peripheral noradrenergic neurons are responsive to appropriate stimuli but release dopamine rather than norepinephrine.

To determine if familial DBH deficiency is due to gross alterations at the DBH locus, we analyzed lymphocytic DNA from DBH deficiency patients and their families, following digestion with Bam HI, Eco RI, and Taq I and subsequent hybridization of a radiolabelled human DBH cDNA. We observed no alterations in restriction patterns in any family members. These findings indicate that DBH deficiency in these families is not due to gross alterations such as insertions or deletions of the DBH alleles. Following digestion with Taq I, we detected an apparent restriction fragment length polymorphism. Further analysis of this polymorphism in additional DBH deficient families will be required to determine if alteration of the DBH gene is present in DBH deficiency.

526.4

PERCEPTUAL-MOTOR TIMING PROBLEMS IN CLUMSY CHILDREN. H. Williams, M.H. Woollacott, & R. Ivry* (SPON: A. Shumway-Cook). Motor Dev. Lab, Univ. of S. Carolina, Columbia, SC 29208, Dept. of PE & HMS, Univ. of Oregon, Eugene, OR, and Dept. of Psych., UCSB, 93106.

A significant aspect of skilled motor performance is the control of the timing of muscular actions. One model of timing control (Wing-Kristofferson) hypothesizes that both a central time-keeper and elements of the peripheral motor control system contribute to variability in the production of isochronous intervals. Since clumsy children are known to have problems with movement coordination, it is possible that their problems are a result of deficits in some aspect of the timing mechanism.

This study examined timing control in clumsy children and tested the Wing-Kristofferson model of repetitive movements in order to determine if their motor coordination problems were specifically associated with either source of variability. Two groups of children classified as normal and clumsy (ages 6-7 and 9-10 yrs) performed tapping and perception of duration and loudness tasks. Results indicated that clumsy children were significantly more variable than normal children in maintaining a set rate of tapping and in accurately judging time intervals. Evidence indicated that the source of timing control problems was in a central time-keeping mechanism, possibly the cerebellum.

526.5

ULTRASTRUCTURE AND IMMUNOHISTOCHEMISTRY STUDIES OF CEREBRAL STRUCTURES FOLLOWING EXPERIMENTAL INFANTILE HYDROCEPHALUS. R.M. Kriebel, K.E. Miller, and J.P. McAllister. Anatomy Depts., Philadelphia College Osteopathic Medicine & Temple University School of Medicine, Philadelphia, PA 19131, and Searle/Monsanto CO., St. Louis, MO 63198.

Hydrocephalus was induced in 4-10 day old kittens by intracisternal injection of kaolin. Hydrocephalic animals were killed at 10-12 days post-kaolin. Ventriculomegaly was confirmed by imaging procedures before the animals were killed. Tissues from cortex (areas 4, 17, 22), basal forebrain and hippocampus were processed for electron microscopy and immunohistochemistry. Deep layers of cortex were severely edematous with marked alterations in neuronal and dendritic morphology including reduction in synaptic contacts. Destruction of neuropil was less in superficial layers, but synaptic numbers were decreased. Dendritic morphology was correlated with observations made on age-matched Golgi impregnated cortex. The decrease in spinous profiles parallels similar observations in Golgi material. Substance P, leucine enkephalin, and ChAT distributions were studied using routine immunohistochemical procedures. Cells in deeper layers gave origin to fiber systems within more superficial layers which survived hydrocephalus but the fibers were compressed in the remaining neuropil.

526.6

MAGNETIC RESONANCE IMAGING IN AUTISM: EVIDENCE FOR A DEFECT OF CEREBRAL CORTICAL DEVELOPMENT. J. Piven*, M.L. Berthier*, S. Starkstein, E. Nehme*, G. Pearlson, S. Folstein*. Department of Psychiatry, The Johns Hopkins University School of Medicine, Baltimore, MD, 21205.

Thirteen autistic individuals and 13 age, sex and IQ matched normal controls were studied by magnetic resonance imaging. All autistic subjects met narrowly defined criteria for autism, and had non-verbal IQ's ranging from 61 to 130. Scans were evaluated by two experienced raters who were blind to the status of the subjects. Five autistic subjects had polymicrogyria, one had schizencephaly and macrogyria, and one had macrogyria only. None of the control subjects had abnormalities of this type on MRI. Polymicrogyria, schizencephaly, and macrogyria are part of a related group of cortical malformations resulting from anomalous development of the cerebral cortex prior to six months gestation. In addition, atrophy of the cerebral cortex was present in four autistic subjects and one control. Structural abnormalities of the cerebral cortex may be common in autism and suggest the possibility that neuronal migration defects may be an important mechanism in the pathophysiology of autism.

526.7

ABSENCE OF BRAINSTEM ABNORMALITY IN STUDY OF AUTISTIC PATIENTS USING MAGNETIC RESONANCE IMAGING. M. Hsu*, E. Courchesne* and G. Press* (SPON: M. Grafe) Dept. of Neurosciences, University of California, San Diego, La Jolla, CA 92093.

In vivo studies using magnetic resonance imaging (MRI) indicate that autism is often associated with developmental hypoplasia of the neocerebellar vermal lobules VI and VII. Recent electrophysiological and neuropathological studies have produced conflicting evidence for brainstem involvement in autism. In our study, we tried to replicate previous findings (Gaffney et al, Biol. Psychiatry, 1988) that the pons was significantly smaller in autistics compared to controls. MRI scans were obtained from 41 autistics (mean age = 17.9 ±1.4, range = 2 to 29 yrs) and 36 normal controls (mean age= 20.6 ± 1.6, range = 3 to 39 yrs) using a 1.5 Tesla magnet (G.E. Signa). The data matrix was 256 x 256 at a field of view of 24 cm yielding a pixel size of 0.94 x 0.94 mm. T1-weighted sequence (TR-600 ms, TE-25 ms) was performed in the sagittal plane on 5 mm-thick sections with 2.5 mm gaps between adjacent sections. Midbrain and pontine sizes were quantified by computer-assisted planimetric analysis using the midsagittal MRI scans. No differences were found between autistics and normals in the midbrain or pons. Our data do not support the suggestion that there are gross anatomical abnormalities in the pons in autism. The discrepancy between our results and the previous finding may be due to different MRI protocols and the use of 10 mm thick sagittal sections in the latter which may result in considerable volume averaging error. However, because the means for the normal groups in the two studies are similar, another possiblity for the difference may be due to the heterogeneous nature of the disorder itself. The smaller number of autistics (N = 13) in the previous study may yield a more homogeneous group thus enabling the detection of subtle differences not found within our more heterogeneous group.

526.8

FOCAL GLIOSIS IN THE AMYGDALA AND TEMPEROPARIETAL ASSOCIATION CORTEX IN A RETARDED WOMAN WITH AUTISM. E. C. Engle*, N.W. Kowall, and A.C. McKee (SPON:V. Knowlton). Neurol. Service & Dept. Neuropath. Mass. Gen. Hosp. Boston MA 02114.

We performed an immunocytochemical study on postmortem brain obtained from a 21 year old retarded woman with clinical features typical of autism who died of pneumonia. Several small cortical blocks and a sample of the amygdala of the left hemisphere were selected for analysis. The remainder of the hemisphere was processed for celloidin embedding and whole brain serial sectioning while the right hemisphere was frozen for future neurochemical studies. Glial fibrillary acidic protein (GFAP) histochemistry showed extensive gliosis throughout all amygdaloid nuclei, CA4 and CA3 fields of hippocampus, and deep layers of cortical samples from Brodmann areas 39,40,41,42,19 (including Wernicke's area). Samples of superior frontal, superior parietal, rolandic, Broca's area, anterior and posterior cingulate, inferior frontal, inferior temporal, and entorhinal cortex only showed subpial gliosis and GFAP positive astrocytes scattered in the white matter. Nonphosphorylated neurofilament immunoreactive pyramidal neurons and parvalbumin immunoreactive local circuit neurons did not appear abnormal in the gliotic cortical regions. The localization of abnormalities in the amygdala and hippocampus supports Baumann and Kemper's previous postmortem study and is consistent with the behavioral abnormalities typical of autism. The affectation of language related cortical areas suggests an underlying cause for the clinical language disorder as well. The distinctive distribution of gliosis in this case, therefore, suggests one possible pathologic correlate of autism.

526.9

MANAGING PERSISTENT REPETITIVE BEHAVIOR: DEVELOPMENTALLY / NEUROLOGICALLY DISABLED ADULTS IN A HORTICULTURAL WORK PLACE. M. C. Wetzel, M. J. Taylor*, and J. M. Lachowicz*. Psych. Dept., Univ. Ariz., Tucson, AZ 85721 and Desert Survivors, 1020 West 22nd St., Tucson, AZ 85713.

Many developmentally disabled persons -- a majority at the Desert Survivors day program -- have recurring, stereotyped behaviors. Central pattern generator neurons may be pathologically active, but any such mechanisms are still highly susceptible to stimulus control and new learning. Daily progress notes and frequent videotape records formed a computer-organized data base. Training interventions based on pattern and rhythm in a given context promoted more harmonious social and work skills, as follows. (1) Rapid, persistent rocking or gestures repeating at 1 or 2 per sec dropped out during organized activity [shovelling; stacking pots; eating]. (2) Staff built rhythmic manual skills [e.g. filling pots with earth], having an orderly onset and completion [cue provided when pot was full], from dysfunctional acts [tossing dirt]. (3) Occasional but strong hitting, rock throwing, or self-abuse also responded when the entire action was guided smoothly to a safer place away from the work crew where its strength could be reduced by stages. These rhythm- and context-appropriate interventions succeeded where traditional behavior modification of isolated responses had failed.

526.10

COMPREHENSION AND EXPRESSION OF AFFECT IN LANGUAGE IMPAIRED CHILDREN. D.A. Trauner, A. Ballantyne,* C.H. Chase and P. Tallal, Depts. of Neurosciences & Psychiatry, Univ. of Ca., San Diego, Ca. 92093.

In addition to their delays in acquiring language, developmental language impaired (LI) children also may have difficulty with the affective intent of communication. We studied 7 LI children and 7 age, sex and IQ-matched controls to determine whether there were group differences in the comprehension and expression of affective intent through spoken language or facial expression. Each child was tested on the ability to 1) identify emotional meaning in verbral phrases; 2) imitate verbal phrases using the same emotional intonations; 3) complete stories using the appropriate spontaneous emotional intonations; 4)select which emotions are conveyed in facial photographs; 5) imitate those facial expressions; and 6) spontaneously produce the appropriate facial expressions in response to stories.

Compared to controls, LI children performed significantly more poorly on tasks involving verbal comprehension and expression (#1,2,3). LI's performed better than controls on production of emotional facial expressions (#5,6). The problem with affective communication in LI appears to be modality-specific. Their superior performance on visual tasks may reflect overcompensation for their auditory deficits through the use of an intact visual communication system.

VISUAL SYSTEM: DEVELOPMENT AND PLASTICITY VI

527.1

LATE POSTNATAL EMERGENCE AND PROTRACTED DEVELOPMENT OF ACETYLCHOLINESTERASE IN MEYNERT SOLITARY CELLS OF THE HUMAN VISUAL CORTEX. I. Kostovic´ and P. Rakic. Sect. of Neuroanatomy, Sch. of Med., University of Zagreb, 41000, Zagreb, Yugoslavia, and Sect. of Neuroanatomy, Yale Sch. of Med., New Haven, CT 06510.

The maturation of Meynert cells in the human striate cortex was studied by use of an AChE histochemical method on postmortem brain tissue obtained from individuals ranging in age from birth to 13 years. During all stages the striate area was sharply delineated by the presence of an AChE reactive band in layer IVC. Although well developed Meynert cells with large pyramidal-shaped somata situated at the interface of layer V-VI are visible in Nissl preparations of the newborn, they do not contain AChE at this stage. Moderate AChE reactivity is first observed in their perikarya at 16 months. By 2 years more than 90% of the perikarya of Meynert neurons are weakly AChE positive. Between 2 and 7 years there is an increase in staining as moderate AChE reactivity extends to proximal dendrites of these cells. By 10 years all Meynert cells are heavily stained and display thick horizontal dendrites. Thus, this solitary class of giant pyramidal neurons expresses AChE reactivity relatively late and their maturation continues through early childhood culminating around puberty. Thus, slow tempo of maturation correlates positively with an extended rate of synaptic elimination (Bourgeois and Rakic, 1983), delayed expression of appropriate laminar distribution of ACh receptors (Rakic et al., 1989), and/or the late development of the cholinergic innervation of Meynert cells in primates which may be related to their connections with medial temporal association cortex. Supported by Yugoslav-U.S. Joint Board.

527.2

THE TEMPORAL RELATIONSHIP OF GABA AND $GABA_A$/BENZODIAZEPINE RECEPTOR EXPRESSION IN NEURONS OF THE VISUAL CORTEX OF DEVELOPING RHESUS MONKEYS D.L. Meinecke and P. Rakic. Sec. Neuroanatomy, Yale Med. Sch. New Haven, CT 06510

An antibody to the GABA transmitter (Incstar Co.) was used in conjunction with a monoclonal antibody (MabE9) (gift of J. Tallman, Yale University) to study the developmental expression of GABA transmitter and $GABA_A$/benzodiazepine (BDZ) receptors in visual cortical neurons of rhesus monkey embryos. The MabE9 recognizes a 50 kD protein which appears to be the benzodiazepine binding γ-2 subunit of the $GABA_A$ receptor complex deduced from cloned cDNAs (Pritchett et al., Nature, 1989, 13: 582). We examined monkeys starting from E41, the time when the cortical plate begins to develop through E165, the end of gestation in rhesus. The first specific and unambiguous labeling with GABA was observed in migrating cells and neurons in the cortical plate at E61. GABA labeled neurons are added to the cortical plate until its completion at E100. In contrast, specific receptor labeling of individual neurons in cortical plate does not become clearly evident until after the cortical plate has fully formed, between E100 and E130. This indicates that in the cortical plate: 1) BDZ receptor maturation follows or maybe induced by GABA expression, 2) BDZ receptor expression is independent of GABA expression, or is related to the onset of synaptogenesis. These possibilities are being further tested by our ongoing in situ hybridization study using cDNA probes to $GABA_A$ receptor subunits and immunoelectron microscopic observations. Supported by NIH grants NS 14841 and NS 2280.

527.3

DEVELOPMENT OF CALCIUM-BINDING PROTEINS IN GABA NEURONS OF MONKEY VISUAL CORTEX. J.F.M. van Brederode, A.E. Hendrickson, K.A. Mulligan & M. Celio* Depts. Biological Structure and Ophthalmology, Univ. Wash., Seattle, WA 98195 and *Anatomy Institute, Univ. Kiel, Kiel, West Germany.

We localized immunoreactivity for two calcium-binding proteins, calbindin (Cal) and parvalbumin (PV) and GABA in neurons of Macaca nemestrina visual cortex. In adult cortex, 90% of GABA neurons contained either Cal or PV, but very few cells contained both. Of the GABA+ neurons, 15% contained Cal and 75% contained PV. Most heavily-labeled Cal+ neurons occurred in layers 2/3 with a few in layers 5/6. PV+ cells are seen in all layers, but the number peaks in layer 4. Diameters of Cal+ cells always were significantly smaller than PV+. PV+ axons run in white matter under striate but not prestriate cortex which can be traced into layer 4. This differential distribution suggests functional differences within the GABA population.

During development, GABA+ neurons are found at fetal (F) 60d, but PV+ cells are first seen at F152d in the deep layers. By postnatal (P) 1-3d, layer 4C is labeled, and by P6 wk PV+ neurons are found in all layers except upper 2 and 1 with PV+ axons in white matter. The adult pattern is not seen until P6 months. In contrast, Cal+ neurons are numerous in layer 1 at F60d and are seen there until after birth. Most cortical layers contain both stellate and pyramidal Cal+ neurons by F136d. Subsequent development has a complex, laminar specific pattern, with the adult pattern and localization only in stellate neurons not found until P6-12 mo. We conclude that the late appearance of PV may correlate with the onset of visual function while Cal must have a role(s) during development as well. (Supported by EY01208,EY04536 &EY07031).

527.4

SUBSTANCE P-LIKE IMMUNOREACTIVITY DURING DEVELOPMENT OF MONKEY VISUAL CORTEX. R.D. Mehra*, A.E. Hendrickson, J.F.M. van Brederode & K. Mulligan, (SPON: M. Koontz). *Dept. Anatomy, All India Inst. Medical Science, New Delhi, India 110029 & Depts. Biological Structure & Ophthalmology, Univ. Wash, Seattle, WA 98195.

We have stained sections of Macaca nemestrina visual cortex with a polyclonal antiserum raised against substance P (PoSPas; Incstar. Corp). Frozen sections of aldehyde fixed cortex were obtained at fetal days (F) 125 and 162 and postnatal (P) 1 day, 3, 9, 20 wk and adult. A monoclonal SPas (Mehra and Hendrickson, Soc.Neurosci.1987) stained stellate cell bodies, dendrites, axonal processes and synaptic terminals. In contrast, the PoSPas stained neuronal perikarya in infragranular layers which exhibited morphological characteristics typical of small pyramidal cells, including apical dendrites which extended to lamina 2. Their cell body diameters ranged between 6.5-12.5 μm. PoSPas also labeled large synaptic terminals which outlined the cell body and primary dendrites of large, unstained neurons which were most common in cortical laminae 3, 4B & 6. Double-labels revealed these cells to be GABA+ and to be the same neurons whose somas are marked by the lectin Vicia villosa (van Brederode etal; Mulligan etal, Soc.Neurosci.1988). This type of SP-like terminal staining was absent at all ages up to P 9 wk; however, SP+ pyramidal cells were seen both at F162 and all postnatal ages. Our study suggests the following: 1) Besides interneurons, some projection neurons of visual cortex may also be using peptides as neurotransmitters/modulators, 2) the activity of a class of large GABAergic neurons may be regulated by a different type of SP-like terminal, and 3) this terminal type appears well after birth. (Supported by EY01208,EY04536 & EY07031)

527.5

[125 I]-APAMIN BINDING TO VISUAL CORTEX OF CAT AND MONKEY: CHANGES IN DISCRETE LOCALIZATION DURING DEVELOPMENT.

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Apamin is a bee venom neurotoxin which selectively blocks some types of calcium-sensitive potassium channels[1]. To study the expression of the apamin receptor during cortical development, [125 I]-apamin (NEN) was applied to 20 µm sections of frozen brain tissue from cats and monkeys of a series of ages. Binding sites in adult cat visual cortex showed a B_{max} of 29 fmol/mg protein and a K_d of 59 pM.

[125 I]-Apamin bound throughout visual cortex in the fetal-day 72 (F72) monkey. In the F152 visual cortex [125 I]-apamin receptor-binding was visible as a broad band in layers 2-3 and as a narrow band at the layer 3/4 boundary, at the layer 4/5 boundary, and in layer 6. This laminar distribution was maintained in visual cortex from all subsequent ages of monkey, with post-natal day 29 (P29) and older ages showing more pronounced binding in layer 6.

In the visual cortex of P1.5 and P10 cats, [125 I]-apamin labelled layer 1 and the layer 5-6 region. In addition, [125 I]-apamin bound to layer 3 in visual cortex of P30 and older cats. The adult distribution of [125 I]-apamin receptor-binding in visual cortex was observed in P60 and older cats, and consisted of binding in layers 1, 3, 5 and 6, with layer 5 showing the most pronounced binding.

Reference: (1) Hugues,M., Romey,G., Duval,D., Vincent,J.P. and Lazdunski,M. (1982) P.N.A.S. U.S.A. 79:1308-1312.

527.6

ONTOGENY OF THE LAMINAR DISTRIBUTION OF ZINC IN THE CAT VISUAL CORTEX.

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The distribution of storage granule zinc in the mammalian brain is generally restricted to the cerebral cortices where, particularly in the hippocampus, it has been implicated in the mechanisms of neural plasticity through its modulation of neurotransmission. Analysis of the ontogenetic localization of zinc in the hippocampus has been reported repeatedly while the neocortex has been studied less intensively. The present work explores the anatomical distribution of zinc in the developing cat visual cortex.

The histochemical localization of zinc in cats of ages ranging from birth (P0) to adulthood was assessed using the selenium method of Danscher (*Histochemistry (1982) 76:281-293*). Briefly, under general anaesthesia cats were administered 20 mg/kg sodium selenate (i.p.) and allowed to survive 40 minutes before perfusing with Sorenson buffer containing 0.5% glutaraldehyde. Selenide-precipitated zinc was visualized by physical development in 16 µm cryostat sections. Selected sections were counterstained with Cresyl violet to establish anatomical boundaries.

Visual cortex in the adult cat was conspicuously distinguished by the absence of zinc staining in layer 4. Layers 1-3 and 5 exhibited intense staining while layer 6 was lightly stained. At P0, the histochemically demonstrable pool of zinc in the visual cortex was confined to a single narrow band above layer 5, deep within the cortical plate. By P10 increased staining was visible within the deeper half of layer 1 and throughout layers 3 & 5. This staining pattern was maintained through P20 and the adult pattern of zinc distribution in the visual cortex was attained by P50.

The complex and transient laminar distribution of histochemically localizable zinc in the developing cat visual cortex is reminiscent of that previously described for localization of neurotransmitter / neuromodulatory receptors. The relationship among these diverse patterns of transient expression are under study.

527.7

PRE- AND POSTNATAL DEVELOPMENT OF NEUROTRANSMITTER RECEPTORS IN MONKEY VISUAL CORTEX

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Using the same in vitro autoradiographic techniques which we have previously used to examine the postnatal development of cat visual cortex, we have now examined the pre- and postnatal development of many neurotransmitter/ neuromodulator (NT/NM) receptors and several ion channels in monkey (Macaca nemestrina) primary visual cortex at 9 ages ranging from F-72 to >1 yr. postnatal. Receptors were labelled by inhibitory NT/NM ligands (GABA-A, GABA-B, BZ), excitatory NT ligands (glutamate, quisqualate, kainate), with ligands for the various NMs (adenosine, CCK, mAChRs (M1 and M2 subtypes), nAChR, and 5-HT), and with ligands for voltage-sensitive Ca++ and Ca-dependent K+ channels.

All receptor populations and ion channels examined were present prenatally and their numbers increased to peak values at different stages of postnatal life. Most of these binding sites showed generally similar distributions to those reported previously for cat visual cortex, but some clear differences were found, notably for nicotinic AChRs labelled with [3H] -nicotine: In cat visual cortex these receptors are localized primarily to layer 4, but in monkey they are most densely distributed in layers 2-3 and 5-6. Many of the receptor and/ion channels examined showed age-dependent expression in different cortical layers just as is found for cat visual cortex.

Such binding sites include: glutamate (labelled with [3H]-glutamate), adenosine, CCK, mAChR, and 5-HT receptors and Ca++ channels.

The present results demonstrate a rich and complex chemical circuitry for monkey visual cortex, one whose characteristics show both similarities and significant differences from cat visual cortex.

527.8

POLYSYNAPTIC, NMDA-MEDIATED POTENTIALS ARE TRANSIENTLY EXPRESSED IN IMMATURE RAT NEOCORTEX.

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We studied the postnatal development of NMDA-mediated activity in rat visual and somatosensory cortical slices between postnatal day (P) 5 and 39. Field potential (FP) responses to orthodromic stimulation of the white matter / layer VI in young ($\leq$P10, n=31) and adult animals ($\geq$P28, n=63) consisted of a short-latency, biphasic wave of 20-30 ms in duration and up to 1.5 mV in amplitude. In 68% of the FPs tested in juvenile rats (P11-20, n=119), low intensity stimuli additionally evoked an oscillatory (30-50 Hz) multiphasic response of variable onset latency (30-220 ms), duration (150-600 ms), and amplitude (up to 0.5 mV). The intracellular correlate of this late population response was a multiphasic, high amplitude, and long-lasting (l)-EPSP, which could be demonstrated in 81% of the juvenile neurons (n=107), and in 3% of the young (n=36) and adult cells (n=61).

Both, the late FP response and the l-EPSP were especially pronounced in upper layers, and could be elicited most reliably at low stimulus intensities (20-70% of the spike threshold), low stimulus frequencies (<0.2 Hz), and at temperatures above 31° C. The late component in the FP (n=4) and the l-EPSP (n=2) was blocked by increasing the extracellular Ca^{2+} concentration. These data indicate that a polysynaptic circuit generated the late responses. Application of APV reversibly blocked or clearly suppressed the long-latency FP response (n=5) and l-EPSP (n=10), indicating that these events were mediated by activation of NMDA receptors.

The transient expression of these polysynaptic, NMDA-mediated events coincides temporally with a period during which LTP is readily induced in rat neocortex (Perkins, A.T. & Teyler, T.J., Brain Res., 439: 222, 1988) and a pronounced susceptibility to epileptiform discharge is present (Hablitz, J.J., J. Neurophysiol., 58: 1052, 1987). These findings suggest that NMDA-mediated polysynaptic events may form an important physiological basis for development of both normal and pathophysiological alterations in cortical excitability during development.

Supported by NIH grants NS06477, NS12151 and DFG grant Lu 375/1-1.

527.9

IMMUNOCYTOCHEMICAL STUDIES OF PROTEIN KINASE C ONTOGENY IN CAT VISUAL CORTEX

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Protein kinase C (PKC) is a major intracellur messenger in brain. It is activated by the inositol lipid hydrolysis and is involved in the phosphorylation of many proteins including myelin basic protein, MAP-2, GAP-43 and various receptors. Many of those proteins play important roles in the development of the central nervous system. In the present work, a polyclonal monospecific antibody against PKC was applied to investigate the ontogeny of the kinase in cat visual cortex with immunocytochemistry. PKC immunoreactivity was always found in layer 2,3,5,6 with lower densities in layers 4 and 1. However, the relative intensity of the staining between layers varied during development. Cells in layers 2/3 showed greater binding than layer 5/6 at 20-40 days postnatal. Thereafter the deeper layers showed greater number of immunoreactive cells. The expression of the kinase in different areas (area 17, 18 and 19) of the visual cortex also varied in young kittens. Many cell types including pyramidal cells were found to be PKC positive. The comparision between the present results and those with [^{3}H]Phorbol ester autoradiography is discussed.

527.10

LAMINAR AND REGIONAL DISTRIBUTIONS OF GABAergic NEURONS IN HUMAN VISUAL CORTEX.

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Previous studies in our laboratory have indicated that there may be plasticity of architectonic boundaries between various regions of the visual cortex in the human neonate. Since gamma-amino butyric acid (GABA) is one of the neurotransmitters that has been implicated in the plasticity of ocular dominance columns in striate (ST) cortex (Hendry and Jones, 1986), we have undertaken a series of studies in the human visual cortex 1) to determine if in the adult there are regional variations in the density and laminar distribution of GABA-containing neurons in ST, parastriate (PARA) and peristriate (PERI) cortical areas, and 2) to characterize the laminar and regional distributions of GABAergic neurons in ST, PARA and PERI cortex at various postnatal ages.

Formalin-fixed human occipital cortices were sectioned at 50µm in the coronal plane using a large-stage freezing microtome. A basic Nissl and the Gallyas silver stain were used for cyto- and myeloarchitectural differentiations of ST, PARA and PERI cortical areas. GABAergic neurons were labelled on mounted sections by the avidin-biotin immunoperoxidase method using antiserum directed against glutamic acid decarboxylase (GAD). CARP software (Biographics, Inc.) was utilized to aid in the visualization of the laminar and regional distribution of GAD-staining somata and/or neurites.

Results from a pilot study demonstrate that layers II and IV manifest the highest density of GAD-staining neurons. In addition, however, and contrary to data obtained in other laboratories in the monkey, our preliminary results suggest that there are regional variations in the density of GABAergic somata in ST, PARA and PERI visual areas of the human brain.

Supported by HD21711 to MCL and the Biological Humanics Foundation.

527.11

RELATIONSHIP OF ACETYLCHOLINESTERASE, CYTOCHROME OXIDASE, AND GENICULOCORTICAL TERMINAL FIELDS IN DEVELOPING AND MATURE RAT VISUAL CORTEX. G.H. Kageyama, M.E. Gallivan*, K.A. Gallardo* and R.T. Robertson. Dept. Anat. and Neurobiol., Univ. of California, Irvine, CA 92717.

Laminar patterns of acetylcholinesterase (AChE) activity in primary visual cortex differs in developing and adult rats. In this study, the pattern of AChE in visual cortex was compared with the distribution of geniculocortical terminals and cytochrome oxidase (CO) activity in developing and mature rats. Infant (P2-P10) and adult rats were injected intraocularly with 1-10% WGA-HRP. After 3-5 days, animals were perfused and sections processed for AChE, CO, or HRP histochemistry.

In visual cortex of both developing and adult rats, intraocular injections of WGA-HRP result in anterograde transneuronal labeling of geniculocortical terminal fields in layers IV and deep III and, to a lesser degree, layers I and VI. In developing animals (P6-P13) the laminar pattern of endogenous AChE reactivity corresponds closely to transported HRP and to endogenous CO reactivity in layers III-IV. In younger animals (P1-P4) the transient AChE is not present, but elevated CO is found in subplate neurons. In visual cortex of adults, AChE is found primarily in layers V, deep IV and I, and differs from the geniculocortical terminal field. These data demonstrate that AChE is found associated with geniculocortical terminals in developing, but not adult, rat visual cortex. Elevated CO, first in subplate neurons and later in neurons of layer III-IV, is more closely associated with development of oxidative metabolism of neurons postsynaptic to geniculocortical projections.

Supported by NSF grant 87-08515 and NIH grant NS 25674.

527.12

The postnatal development of horizontal intrinsic connections in the cats striate cortex. J.Lübke*and K.Albus(SPON:P. Wahle).Dept.Neurobiol.MPI Biophys.Chem.,3400Göttingen,FRG.

Intrinsic horizontal connections were visualized by means of retro-and anterograde axonal transport of HRP and WGA-HRP. Injections of WGA-HRP label around the injection site distinct patches consisting of somata and axon terminals. The number of patches may vary considerably even within the same cortex. The patches have diameters between 200-500µm and maximal distances between a patch center and the outer margin of the diffusion zone around an injection are about 3mm. No significant differences in the number and the size of patches and in maximal distances of patches from the injection site were found for developmental stages between one month and one year postnatally; patches seemed to be less developed in the one week old cortex. In cases with injections of HRP, at one month and one year postnatally,labeled axons and somatawere seen up to 3mm distant from the injection site. The patches in these cases consisted of labeled axons and terminal-like structures and -in contrast to the WGA-HRP cases- contained only few labeled somata. This discrepancy between the WGA-HRP and the HRP data might be explained by transneuronal transport of WGA-HRP which would result in greater numbers of labeled somata within the patches.Our results so far indicate that intrinsic horizontal connections are adult-like at one month postnatally.We have not seen exuberance, at the end of the 1st postnatal month, of intrinsic connections (Luhmann etal.,EBR1986,63:443-448).

527.13

WHOLE-CELL VOLTAGE-CLAMP ANALYSIS OF NEUROTRANSMITTER SENSITIVITY AND SYNAPTIC CURRENTS IN DIFFERENTIATING CORTICAL NEURONS. M.G. Blanton and A.R. Kriegstein. Dept. of Neurology, Stanford University Medical Center, Stanford, CA 94305-5300.

To analyze the appearance of neurotransmitter responsiveness and maturation of synaptic circuits during development of cerebral cortex, we have recorded from neurons of embryonic *Pseudemys scripta* turtles *in vitro*. The cortex could be unfolded, with thalamocortical and corticocortical connections intact, and superfused with turtle saline for recording. Recordings were obtained by applying the whole-cell voltage-clamp technique to neurons from stages 16 (early morphogenesis) to 26 (hatching).

Morphologically identified cortical pyramidal cells expressed multiple receptor subtypes for both the excitatory neurotransmitter glutamate and the inhibitory transmitter γ-aminobutyric acid (GABA), prior to the presence of detectable synaptic currents. Infrequent spontaneous postsynaptic currents appeared at stage 18. Specific thalamic stimulation at this stage evoked synaptic currents mediated by non-N-methyl-d-aspartate (non-NMDA) glutamate receptors, and at stage 19, by $GABA_A$ receptors. Stimulation of caudorostral intracortical connections activated, in addition, a $GABA_B$-mediated current. Early synapses on cortical neurons therefore activate specific subpopulations of neurotransmitter receptors present on postsynaptic cell membranes.

527.14

DEVELOPMENT OF CORTICOTECTAL AXON ARBORS LABELED WITH PHASEOLUS VULGARIS LEUCOAGGLUTININ (PHAL). K. Lord Plummer*, and M. Behan. Center for Neuroscience and Dept. of Comparative Biosciences, University of Wisconsin, Madison, WI 53706.

We are interested in determining how individual corticotectal axons arising from neurons in striate cortex develop. Small iontophoretic injections of PHAL were made in the cortical representation of area centralis in cats. Injections at 2, 4, and 12 weeks of age resulted in labeling corticotectal axons which could be reconstructed from their entry into the midbrain at the level of the rostral brachium to their terminal arbors in the superficial layers of the colliculus.

The retinotopic organization of the corticotectal projection to the superficial layers of the colliculus remains grossly unchanged during postnatal development. However individual corticotectal axon arbors demonstrate substantial developmental changes, both progressive and reductive in nature. At 2 weeks of age corticotectal axon collaterals elaborate *en passant* swellings, a few single terminal boutons attached by thin stalks, and numerous growth cones. By 4 weeks of age only a few structures resembling growth cones are observed. Between 4 and 12 weeks *en passant* and terminal boutons become more numerous. At 12 weeks of age corticotectal axon arbors exhibit a more complex branching pattern, and a few terminal clusters of boutons not observed at 2 and 4 weeks. Estimates of the volume of the terminal arbor indicate a decrease in size from 2 to 12 weeks. The growth of the superficial division of the colliculus was assessed by measuring its volume at different postnatal ages, and was found to undergo a 2-fold increase from newborn to adult. These results taken together indicate an overall reduction in the size of the terminal arbor with a concommitant increase in the complexity of collateral branching and terminal arbor specializations. (Supported by NIH Grant EYO4478).

527.15

EXTENSION, BRANCHING AND REGRESSION OF VISUAL CORTICOSPINAL AXONS IN RATS B.S. Reinoso and D.D.M. O'Leary, Dept of Neurosurgery, Washington Univ. Sch. Med, St. Louis, MO 63110.

Layer 5 neurons in the visual cortex of rats transiently extend axons through the pyramidal tract and into the spinal cord (Stanfield, O'Leary & Fricks, 82, Nature 298:371). We report here on the development of these axons anterogradely labeled in vivo with the high resolution axon tracer, DiI. Small injections of DiI were made into the visual cortex of newborn to P6 Sprague Dawley rats. 2 - 3 days later, the pups were perfused with 4% paraformaldehyde. Sagittal and horizontal sections of the brain and spinal cord were cut, respectively, on a vibratome and analyzed with RITC fluorescence optics. Injection placements were confirmed in alternate sections processed for AChE histochemistry and Nissl staining. Only cases with injections confined to primary visual cortex (area 17) are considered. The extension of visual corticospinal axons is staggered, i.e. the number of labeled axons decreases distally. On P2, the most distal axons have reached upper cervical spinal cord. The axons attain their caudalmost extent, upper thoracic cord, on P4. The labeled axons are on the side contralateral to the injection, mostly in the dorsal funiculus, their main pathway. At these ages, few axons are found in the spinal gray adjacent to the dorsal funiculus, but some axons grow in the intermediate corticospinal tract. After P4, the caudalmost axons are seen at progressively more rostral levels: cervical cord at P6-P8, spino-medullary junction at P10-12, and by P16, axons could not be seen beyond the pons. Over P4 - P10, collateral branches develop to the medullary nuclei, the inferior olive and dorsal column nuclei, and occasional axons in the dorsal funiculus extend collaterals into the spinal gray. These findings indicate that corticospinal axons do arise from visual cortex, and that they appear to regress progressively from their distal ends. Since some of these axons form collateral branches to targets appropriate for corticospinal axons, their selective loss is not due to a failure to enter terminal zones. (Supported by NEI grant EY07025 & The McKnight Foundation)

527.16

DEVELOPMENT OF THE CORTICOTECTAL PROJECTION IN THE NEONATAL RAT. F.A. Richburg and M.A. Kirby, Departments of Pediatrics and Anatomy, School of Medicine, Loma Linda University, Loma Linda, CA 92350.

One of the focal points in development concerns the topographic order of afferents during the initial phase of axon ingrowth. To explore this we have examined the density and distribution of labeled cortical cells following placement of discrete deposits of tracers into the superior colliculus (SC) of neonatal and adult rats.

Small deposits of red and green latex microspheres (0.02-0.1ul) were placed into the SC using glass micropipettes (tip diameter 25-35um) in rats ranging in age from birth to adulthood. Following a 48hr survival the animals were deeply anesthetized and perfused with saline followed by a 10% formal-saline solution. The location and density of labeled cortical neurons were then plotted for computer reconstruction.

Confirming the observations of Thong and Dreher (1986), the earliest labeled cells were found at postnatal day three (P3). Between P4-P9 the number of labeled cells increased substantially occupying widespread cortical regions. These decreased in number and area after P9, with few labeled cells observed outside of visual cortical areas by P14. At each age examined (>P5) an area of densely labeled ganglion cells was observed in a restricted portion of primary visual cortex, corresponding to the topographic location of the SC deposit site. We conclude that the early ingrowth of cortical fibers to the SC, although widespread and exuberant, does possess a rough topographic order (based on peak cell density and location). Refinement of this projection occurs during the second and third postnatal weeks, establishing the mature pattern by P19.

527.17

BILATERAL CORTICOTECTAL PROJECTION FOLLOWING NEONATAL CEREBRAL HEMISPHERECTOMY IN THE CAT. D.A. Hovda and J.R. Villablanca. Depts. of Psychiat. & Anat., Mental Retard. Res. Ctr., UCLA Sch. of Med., Los Angeles, CA 90024.

Following neonatal cerebral hemispherectomy cats show sparing of the contralateral visual field. Considering the current understanding of the "Sprague Effect" we wished to determine if these animals show a reorganization of the corticotectal projection arising from the primary visual cortex (areas 17 & 18). WGA-HRP (5%/0.9% saline; .3-.5 μl) was injected (1 mm deep) into the left (ipsilateral to the lesion) superior colliculus (SC) in 6 adult cats (2-intact; 2-neonatal-lesioned, 7 DOA; 2-adult-lesioned). 48 h later the animals were processed for combined TMB/DAB histochemistry and the visual cortex was examined for retrograde labeling in 100 μm thick, coronal, frozen sections taken every 400 μm with cells counted in every third section. Intact animals showed no evidence of a bilateral projection. Adult hemispherectomized cats showed only a few cells (<10) restricted to the anterior-most aspect of the 17-18 boarder. In contrast, neonatal lesioned animals showed as many as 95 cells located throughout the anterior and posterior extent of areas 17 & 18. These results indicate that following neonatal cerebral hemispherectomy the intact visual cortex projects bilaterally to the SC and suggests that this may underlie the partial sparing of the visual field.(Giannini Found.,USPHS HD-05958,RO1 NS25780, HD-04612)

527.18

THE DEVELOPMENT OF CALLOSAL CELL DISTRIBUTION IN THE VISUAL CORTEX OF NEONATAL RATS.

C.S. Hermit, K.M. Murphy*, and R.C. Van Sluyters* (SPON: T.E. Cohn), Department of Physiology and School of Optometry, University of California, Berkeley 94720

In adult rats visual callosal cells in layers II-Va of the cortex are distinctly concentrated at the border between areas 17 and 18. The distribution of visual callosal cells is fully mature as early as postnatal day (PND) 12. However, features of the mature pattern are specified at earlier ages. In an effort to gain a better understanding of the factors responsible for specifying the callosal pattern, we examined the distribution of visual callosal cells during the first week of life. Bulk injections of a persistent, retrogradely transported, fluorescent tracer (rhodamine-conjugated latex microspheres) were made into the posterior neocortex on PND 1, 6, or 12, and injected pups were allowed to survive for various periods of time prior to perfusion. Injections were aimed at the white matter, to ensure that all cells with an axon crossing the callosum were labelled. Assessment of the radial and tangential organization of callosally labelled cells in these pups indicates that adult-like features of the mature callosal pattern are present as early as PND1. Cells that send axons across the callosum on PND1 have completed their migration into the cortical plate and lie in the deep layers, in their final radial positions. The radial distribution of callosal cells is mature by about 6 days of age -- approximately a week prior to the tangential distribution. It is possible that maturation proceeds more slowly in the tangential plane because of factors that are extrinsic to the developing neocortex.

527.19

VISUAL CALLOSAL PROJECTIONS IN THE STRIATE CORTEX OF THE RABBIT FOLLOWING STROBE-REARING. A.M. Grigonis and E.H. Murphy. Departments of Anatomy, Hahnemann University, Philadelphia, PA 19102-1192, and Medical College of Pennsylvania, Philadelphia, PA 19129.

The cell distribution of the corpus callosum (CC) projection in the rabbit is exuberant at birth and its tangential extent fills most of mediolateral area 17. This exuberant projection retracts during normal development to become restricted to the 17/18 border in adults. We have previously shown that disruption of normal activity of the visual system during development results in an increase in the tangential extent of the CC cell distribution in the adult. In the present study we examined the effects of altered activity by rearing animals in stroboscopic illumination. Seven Dutch-Belted rabbits were raised under strobe-illumination (8 Hz, 10 μsec flash duration) for 24 hours/day beginning on day 5. After five weeks, multiple injections of HRP (Sigma VI, 20% in H_2O) were made (12 μl) throughout one entire visual cortex. Animals were perfused 24 hours later and the brains were cut and reacted with TMB. Strobe-rearing resulted in a significant increase in the density of the CC cell distribution compared to the CC cell distribution in normally reared adult rabbits, although the tangential extent of the CC projection was normal. The results suggest that synchronous activation by retinal ganglion cells induced by strobe light may enhance the maintenance of callosal projections in the normal callosal zone, while patterned visual experience eliminates exuberant projections outside the normal callosal zone. Supported by grant NS26989.

527.20

VISUAL CALLOSAL DEVELOPMENT IN STRABISMIC CATS.

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In adult rodents and cats visual callosal cells are concentrated in the region of the border between areas 17 and 18. Previous data from congenitally anophthalmic rodents, binocularly enucleated rats & kittens, monocularly enucleated rats, & Siamese cats show that callosal development is guided by the pattern of cortical retinotopy. Reports that callosal connections are widespread in strabismic cats are at odds with this idea since retinotopy appears normal in these cats. For this reason we re-examined the distribution of callosal cells in cats reared with esotropia or exotropia.

Bulk injections of the tracer horseradish peroxidase (HRP) were made at 20-30 sites scattered evenly across area 17 in one hemisphere of 12 adult cats that were either reared normally or made surgically strabismic at the time of eye opening (4 normally reared, 4 exotropic, and 4 esotropic). To compare the tangential distributions of callosal cells in normally reared and strabismic cats, HRP-labeled cells of the opposite hemisphere were counted in camera lucida tracings of coronal sections at 960μm intervals. The region of tissue analyzed ranged in length from 11.5 to 18.7mm.

In each group callosal cells are densely concentrated in the region of the 17/18 border. Also, cells do not appear abnormally widespread in strabismic cats. A 1mm wide region centered on the 17/18 border contains 37%, 43%, & 43% of the labeled cells in normally reared (N), exotropic (Exo), and esotropic (Eso) cats, respectively. The 2mm wide region of area 17 between 0.5mm & 2.5mm medial to the 17/18 border contains 31% (N), 26% (Exo), & 24% (Eso) of the cells. The remainder of area 17 contains 4.0% (N), 1.7% (Exo), & 1.4% (Eso) of the cells.

This lack of a significant difference between the overall distribution of visual callosal cells in normally reared and strabismic cats indicates that the ontogeny of the interhemispheric cortical visual pathway in the cat is not susceptible to the effects of ocular misalignment during postnatal development.

527.21

CORPUS CALLOSUM CONNECTIONS IN VISUAL CORTEX OF NEONATAL CAT EXAMINED BY DOUBLE-LABELING WITH A CARBOCYANINE DYE (DiI) AND FLUORESCENCE IMMUNOREACTIVITY. A.J. Elberger, Anatomy & Neurobiology, Univ. Tennessee Memphis, TN 38163.

DiI can be applied to aldehyde-fixed tissue to label neuronal elements retrogradely and anterogradely, and is visualized with a fluorescent light source using a filter appropriate for rhodamine. To define corpus callosum neurons within visual cortex of the neonatal cat, and to identify possible neurotransmitters of callosal neurons or of neurons in contact with callosal neurons, DiI-labeled tissue was subjected to immunohistochemical procedures.

Crystals of DiI were placed in the midsagittal corpus callosum in slabs of neonatal cat brain to label callosal cell bodies and processes. After several weeks in the dark, slabs were vibratomed coronally at 50 μm thickness. Free-floating sections were reacted with antisera to Glutamate (GLU), GABA, Somatostatin (SS) or Vasoactive Intestinal Polypeptide and then with an FITC-conjugated secondary antibody. Sections were mounted on glass slides and viewed for both DiI and neurotransmitter label. Antisera labeled cell bodies and processes, and each antiserum had a unique labeling pattern. DiI-labeled callosal cells were double-labeled with antiserum to GLU and SS but not GABA. In some cases, antiserum-labeled neuronal elements were apposed to DiI-labeled callosal elements. Double-labeling with DiI and immunofluorescence provides a sensitive method for studying neonatal tissue. Supported by EY06362 and BRSG-RR05423.

527.22

THE EFFECT OF NEONATAL HYPOXIA-ISCHEMIA ON THE DISTRIBUTION OF VISUAL CALLOSAL PROJECTIONS IN THE CAT. B. Miller*, S. Nioka*, A. Zaman*, B. Chance, and B.L. Finlay. Dept. of Psychology, Cornell University, Ithaca, NY, 14853, and Dept. of Biochemistry and Biophysics, University of Pennsylvania, Philadelphia, PA, 19104.

Hypoxic-ischemic insult in the perinatal period produces diffuse and focal neuropathology. The organizational consequences of this neuropathology are unknown. One feature of early brain development is the exuberance and retraction of transient axonal projections. In this experiment we examine the effect of hypoxia-ischemia on developmental axon retraction . In the neonatal kitten, the entire visual cortex projects through the corpus callosum to the contralateral hemisphere. In adults the distribution of callosal cells is restricted to a region near the border between area 17 and area 18. Several manipulations can preserve some of these transient axons, such as cortical damage and an unusual visual environment.

At 14 days of age two cats were subjected to hypoxia-ischemia by bilateral carotid artery ligation. The degree of hypoxia in the neocortex was monitored by phosphorus NMR to measure high energy phosphorus metabolites to determine metabolic states. Every week for 2 months and then bi-weekly up to 3 months of age, the cats were given neurological examinations consisting of locomotor, superficial and deep reflex and sensory nerve function, including the visual cliff task.

After the cats reached 3 months of age, multiple HRP injections were made into the visual cortex of one hemisphere. The tangential extent of retrogradely labeled callosal cells was measured from the 17/18 border, and the number of cells was counted in 200um increments. The distribution of callosally projecting cells was compared between the 2 hypoxic-ischemic animals and 2 controls.

The results suggest that the hypoxic-ischemic episode induced a complex reorganization of callosally projecting cells in visual cortex, with substantial inter-animal variability. The tangential extent of callosal cells in the caudal half of area 18 was decreased. The number of callosally projecting cells was not affected. SUPPORTED BY NIH RO1NS19245 and NS22881.

527.23

REORGANIZATION OF VISUAL CALLOSAL PROJECTIONS AFTER EARLY THALAMIC LESIONS IN THE GOLDEN HAMSTER. B. Howard*, B. Miller*, B. L. Finlay (SPON: E. Adkins-Regan) Department of Psychology, Cornell University, Ithaca, New York, 14853.

Various manipulations have been shown to preserve early exuberant callosal projections, including cortical damage and unusual visual environments. During early development the entire tangential extent of the visual cortex projects through the corpus callosum to the contralateral hemisphere. The mature callosal projection is restricted to regions near the border between primary and secondary visual cortex. Here we test whether visual callosal projections originate from a greater tangential extent when thalamic input has been removed from the contralateral visual cortex.

On the day of birth hamster pups received unilateral electrolytic lesions to the visual nuclei of the thalamus. The dorsal lateral geniculate nucleus (LGd), and sometimes the lateral posterior (LP) and lateral (L) nuclei were damaged. At 30 days of age the animals received a unilateral implant of HRP in the visual cortex ipsilateral to the damaged thalamus. The distribution of retrogradely labeled cells in the contralateral cerebral hemisphere was plotted. These animals were compared to normal adult animals with comparable HRP injections.

An increase in the tangential extent of callosally projecting cells in striate cortex was observed in every animal with early thalamic damage. Thus, thalamic damage, like other manipulations which alter patterns of neuronal distribution or activity, can modify the organization of callosal projections.

Supported by NIH R01 NS19245.

HYPOTHALAMIC-PITUITARY-GONADAL REGULATION III

528.1

NEUROPEPTIDE K INHIBITS PROGESTERONE-INDUCED LH SURGE IN ESTROGEN-PRIMED OVARIECTOMIZED RATS. S.P. Kalra, M.G. Dube* and A. Sahu, Dept. OB-Gyn, Univ. Fla., Gainesville, FL, 32610.

Neuropeptide K (NPK), a novel 36 amino acid tachykinin has been localized in several sites of the rat hypothalamus including those areas previously implicated in the control of gonadotropin secretion. NPK receptors also have been visualized in the rat brain. Intracerebroventricular (icv) NPK promptly suppressed LH secretion in ovariectomized (ovx) rats to the range seen after estrogen treatment. These studies suggested that NPK may act as an inhibitory neuropeptide. We have examined the effects of icv and peripheral (i.v.) injections of NPK on the LH surge induced by progesterone (P) in estradiol benzoate (EB)-primed ovx rats. One week after implantation of icv cannula and ovariectomy rats were primed with EB (30 μg/rat) at 1000 h (day 0). On day 1 rats received intrajugular cannulae and on day 2 blood samples were withdrawn at 1000 h before P injection (2 mg/rat) and at hourly intervals from 1300 to 1800 h for LH measurements by RIA. Either one injection of NPK (0.5 or 1.25 nm in 3 μl saline) at 1300 h just before the onset of LH surge or two injections at 1300 and 1500 h inhibited the P-induced afternoon LH surge which occurred in control rats receiving icv saline only. However, i.v. NPK (0.5 or 1.25 nm) at 1300 h failed to suppress the P-induced LH surge. Finally, to assess the specificity of NPK action, the effects of neurokinin A (NKA), another member of the tachykinin family comprised of a 10 amino acid sequence at the carboxyl terminal of NPK, were studied. NKA injection (1.25 nm, icv) at 1300 h produced partial suppression of the LH surge (3/5 rats). These studies show that NPK acts centrally to inhibit the steroid-induced LH surge and it is relatively more effective than NKA. The profound inhibitory effects of NPK on LH release in ovx and steroid-primed ovx rats imply that hypothalamic NPK may serve as a mediator of inhibitory feedback effects of steroids on LH release. (Supported by NIH HD 08634).

528.2

GALANIN STIMULATES LHRH SECRETION FROM ARCUATE NUCLEI-MEDIAN EMINENCE FRAGMENTS IN VITRO: INVOLVEMENT OF AN α-ADRENERGIC MECHANISM. F.J. Lopez*, and A. Negro-Vilar (SPON:R.McLamb) Reproductive Neuroendoc. Section, Lab. of Mol. Integrat. Neurosci., NIEHS, Res. Tri. Park, NC 27709

Galanin (GAL), a peptide distributed in the gut and brain, is strategically located to regulate hypothalamic-pituitary function. We evaluated the effects of rat GAL on LHRH and PGE_2 release from arcuate nuclei-median eminence (AN-ME) fragments incubated in vitro in Krebs-Ringer-Bicarbonate buffer. LHRH and PGE_2 levels in the medium were measured by RIA. The addition to the medium of rGAL [1-500 nM] increased the release of both LHRH and PGE_2 in a concentration-dependent manner. The ED_{50} was approximately 55 nM and 80 nM for LHRH and PGE_2, respectively. rGAL-induced LHRH and PGE_2 release are coupled, since inhibition of PGE_2 synthesis with indomethacin (10 μM) completely blocked rGAL-induced LHRH release. In addition, an active catecholaminergic input was needed for obtaining the stimulatory effect of rGAL, since the addition of the α-adrenergic blockers, phentolamine or prazosin, impaired the ability of rGAL to release both LHRH and PGE_2. In summary, rGAL stimulates LHRH and PGE_2 release from AN-ME fragments in vitro in a dose-dependent fashion. Such an effect is blocked by both indomethacin and α-adrenergic blockers, indicating that rGAL stimulation of LHRH secretion requires activation of α-adrenergic receptors and involves PGE_2 as an intracellular mediator.

528.3

ANTISERUM TO NEUROPEPTIDE Y (NPY) SUPPRESSES LH IN RABBITS. ALAN H. KAYNARD*, K.-Y. FRANCIS PAU* AND HAROLD G. SPIES. OREGON REGIONAL PRIMATE RESEARCH CENTER, BEAVERTON, OR 97006.

In the estrous rabbit, NPY has stimulatory actions on both luteinizing hormone (LH) secretion and gonadotropin-releasing hormone (GnRH) release. Similar actions of NPY on the GnRH/LH axis have been shown in the rat, including the ability of peripheral NPY-antiserum (NPY-Ab) administration to inhibit the proestrus LH surge. The present study examined the role of NPY in maintaining tonic, basal LH release in the rabbit. Eight intact (INT) rabbits had 3rd cerebroventricular (3-IVT) cannulae and venous catheters implanted and were subjected to 8 h of blood sampling at 15 min intervals. 3-IVT infusion of 1 ml of either normal rabbit serum (NRS) or NPY-Ab (raised in rabbits against human NPY) was begun after 2 h of basal sampling and was continued for 6 h. A matching i.v. dose of NRS or NPY-Ab was administered at the start of the 3-IVT infusion. All rabbits received both NRS and NPY-Ab treatments two weeks apart in a Latin-Square design. These treatments were repeated in 4 rabbits 2 weeks after they were ovariectomized (OVX). Administration of NPY-Ab significantly ($p<0.05$) suppressed plasma LH after 165 min in INT rabbits and 75 min in OVX rabbits. After 4 h plasma LH was reduced by 58% and 79% in INT and OVX respectively (Table). Treatment with NRS had no effect on LH in either group (Table). FSH levels were not affected by either NRS or NPY-Ab. These results clearly indicate that in both INT and OVX does, endogenous NPY is in-part responsible for maintaining basal, tonic LH secretion. Since NPY-Ab was simultaneously administered centrally and peripherally, we cannot determine if NPY is acting via alterations in hypothalamic activity or via direct action on the anterior pituitary. Spt'd by NIH HD16631, HD07044. Tabular Data: LH pg/ml

Group	n	Pre	2h	4h	6h
INT NRS	8	102±19	90±41	86±25	78±18
INT NPY-Ab	8	93±16	64±11	39±5	36±5
OVX NRS	3	841±274	565±398	662±368	637±311
OVX NPY-Ab	4	805±142	200±66	172±54	192±65

528.4

THE EFFECT OF SEQUENTIAL CASTRATION AND TESTOSTERONE REPLACEMENT THERAPY ON IN VIVO LHRH SECRETION IN THE MALE RAT. R.L. Pickle and V.D. Ramirez, Dept. of Physiology and Biophysics, Univ. of Illinois, Urbana, IL 61801.

The application of push-pull perfusion (PPP) to the adenohypophysis is a means whereby LHRH can be measured at its site of action, providing an index of summed hypothalamic release of this peptide in freely behaving animals. Moreover, this technique allows repetitive sampling of an individual animal over a protracted period of time, under different hormonal circumstances. The present study applies this strategy toward determining the effect of castration and subsequent testosterone (T) replacement on the in vivo release of LHRH.

Young adult male rats (n=4) were stereotaxically implanted with a push-pull cannula directed toward the adenohypophysis and perfused 10d later as intact animals. These animals were then castrated and perfused a second time 14d post castration. A third perfusion was performed following 7d T replacement using a 30 mm Silastic capsule. PPP lasted 4 hr with samples continuously being collected at 10 min intervals for subsequent LHRH RIA. Significant differences were observed in the mean release of LHRH (pg/10 min) between intact (1.85 ± 0.15) and castrate (3.56 ± 0.10) conditions, and interestingly between castrate and T replacement (5.82 ± 0.24) conditions. Furthermore, these changes appear to be due to an increase in basal release rather than in pulse amplitude or frequency.

528.5

17-β ESTRADIOL- (E_2) INDUCED EFFECTS ON THE SECRETION OF LUTEINIZING HORMONE RELEASING HORMONE (LHRH) AND β ENDORPHIN (β-EP) IN RAT PITUITARY PORTAL BLOOD FOLLOWING A CUTE OVARIECTOMY (OVEX) ON DIESTRUS II . S.F. Krautschy*, D.K. Sarkar (SPON: L. DePaolo) Dept. VCAPP Washington State University. Pullman, WA 99164

Although indirect evidence strongly supports a role for β-EP in the feedback action of E_2 on LHRH secretion direct effects of E_2 on time-dependent changes in β-EP and LHRH secretion remain controversial. 4-day cyclic female rats were OVEX at 0800 h on diestrus II after which 10 μg of E_2 or 0.1 ml of oil sc were administered at 1000 h. On the subsequent day of expected proestrous animals were anesthetized with Saffan, the pituitary stalk surgically exposed, the pituitary removed by aspiration and pituitary portal blood samples were collected at 15 minute intervals from 1000 - 1400 h or from 1400-1800 h. These samples were measured for LHRH and β-EP by RIA. In control animals there was an increase in basal levels and decrease in pulse duration of LHRH during the afternoon compared to the morning. There was also a marginal increase in mean levels of LHRH which may represent initial changes associated with removal of negative gonadal steroid feedback. In response to E_2 there was an inhibitory effect on LHRH secretion in the morning as compared to the afternoon hypersecretion of LHRH (mean and basal levels, secretion rate, amplitude, and frequency). This was in contrast to the effects of E_2 in the long-term OVEX model that we observed previously in which no inhibitory phase was observed. Despite considerable between-animal variation in mean levels of β-EP, there was a consistent effect of treatment on pulse frequency of β-EP which we also observed in the long-term OVEX rat. There was a significant stimulation of β-EP pulse frequency during the morning period in E_2-treated animals compared to the morning period of control animals. In E_2-treated animals there was a suppression of pulse frequency of β-EP and prolongation of pulse duration of β-EP in the afternoon compared to morning. These results suggest that an E_2-induced alteration in β-EP pulse frequency is inversely associated with LHRH secretion and may mediate steroid feedback action of E_2 on LHRH secretion.

528.6

ESTRADIOL EFFECTS ON PLASMA LH RELEASE IN NORMAL MICE AND HYPOGONADAL (HPG) MALE MICE WITH PREOPTIC AREA (POA) GRAFTS. M.J. Gibson and A.J. Silverman. Dept. of Medicine, Mount Sinai Sch of Medicine, New York, NY 10029.

Hpg mice, genetically unable to produce GnRH, respond to POA grafts which contain GnRH cells with increased gonadotropin release and gonadal development. As part of a series of studies examining steroid hormone feedback in hpg mice with POA grafts(HPG/POA), normal and HPG/POA male mice received 1.0ug 17 beta-estradiol (E2) implants at the time of castration. One week later, plasma LH was elevated in both groups, in contrast to results in similarly treated females where plasma LH remained at preovariectomy levels. To study differences in sensitivity to E2 priming, male and female mice were gonadectomized and given Silastic capsule implants of sesame oil (SO), or 10, 1.0 or 0.1ug E2/SO for one week. Plasma LH was suppressed to pregonadectomy levels in females by 10 and 1.0 ug E2 implants and in males only by 10ug E2, suggesting that normal male mice are less sensitive than females to E2 feedback. Pituitary responsivity to GnRH (100ng, iv) was evaluated via intrajugular catheters in normal and HPG/POA castrate male mice with SO or 1.0ug E2 implants. Plasma LH peaks after GnRH administration were +5 min in SO-treated mice and delayed in E2-treated mice to +15 min, but the lack of other significant effects of E2 on pituitary responsivity is consistent with a central action of E2 on GnRH secretion.

528.7

METHOXAMINE (MTX), AN α_1-ADRENERGIC AGONIST, STIMULATES IN VIVO LHRH RELEASE IN PRE- AND PERIPUBERTAL FEMALE RHESUS MONKEYS. *A.C. Gore & E. Terasawa.* Neurosci. Training Prog. & Wis. Reg. Primate Res. Ctr., Univ. Wisconsin, Madison, WI 53715.

Previously, we have reported that norepinephrine (NE) plays an important role in the control of pulsatile LHRH release in adult ovariectomized monkeys. Since the onset of puberty is characterized by increases in pulsatile LHRH release, it is possible that developmental changes in the NE neuronal system contribute to the increases in LHRH release associated with puberty. In order to distinguish whether changes in NE release or NE receptor dynamics occur during puberty, we tested the effects of MTX on LHRH release in prepubertal (17-20 mo) and peripubertal (32-48 mo) monkeys. Using an *in vivo* push-pull perfusion method on conscious monkeys, perfusates were collected on ice in 10 min fractions for 12 h. After 2 h of control sampling, MTX (10^{-5} or 10^{-8} M) or vehicle was infused for 10 min through the push cannula at 1-1/2 h intervals. LHRH levels in perfusates were estimated by RIA. LHRH release during the 20 min periods before and after MTX or vehicle administration was compared. *Results*: MTX increased LHRH release 6-11 fold in prepubertal monkeys, and 2.5-5 fold in peripubertal monkeys. In contrast, vehicle infusion resulted in no change in LHRH release. To our knowledge, this is the first demonstration that the LHRH neurosecretory system in prepubertal monkeys is capable of responding to α_1-adrenergic stimulation with increases in LHRH secretion. The data further suggest that prior to puberty, α_1-adrenoceptors are already present on LHRH neurons or on interneurons affecting LHRH release. Since the LHRH neurosecretory system can respond to α_1-adrenergic stimulation prepubertally, it is possible that the pubertal increases in LHRH release are due to developmental changes in the neurosecretory pattern of NE release.

528.8

OCCURRENCE OF SLOW AND PROLONGED LHRH PULSES IN OVARIECTOMIZED FEMALE RHESUS MONKEYS. M. Gearing and E. Terasawa. Neuroscience Training Prog. and Wisconsin Reg. Primate Res. Ctr., Univ. of Wisc., Madison, WI 53715.

The push-pull perfusion technique has been used extensively in our laboratory to measure the release of luteinizing hormone releasing hormone (LHRH) in conscious ovariectomized female rhesus monkeys. In most cases, LHRH release from the stalk-median eminence consists of pulses of uniform amplitude with a sharp rising phase, superimposed on a stable baseline level of LHRH; these pulses typically have an interpeak interval of 40-50 min and a duration of approximately 40 min, with peak levels being attained within 10-20 min. In a few cases, though, peaks of typical duration and latency were separated by prolonged interpeak intervals of 2-3 h. In contrast to the pattern described above, the spontaneous LHRH release pattern observed in 4 of the 19 monkeys was markedly different. In these 4 monkeys, LHRH pulses were superimposed on a slowly fluctuating baseline. These wavelike fluctuations had a period ranging from 2 to 6 hrs, an average duration of approximately 160 min, and a trough-to-peak time of 40-140 min. The patterns of LHRH release observed in these monkeys did not appear to be related to the ages of the monkeys, length of time after ovariectomy, or sampling location within the stalk-median eminence; thus their physiological significance is unclear. Since it has been shown that the shape of a LHRH pulse is an important factor in the amount of LH released by the pituitary, the LHRH release profiles observed in the present study may add a new dimension to the investigation of the LHRH pulse generator and its relationship to LH release. (Supported by NIH grant HD15433.)

528.9

LUTEINIZING HORMONE-RELEASING HORMONE (LHRH) NEURONS FOLLOWING EXPOSURE TO GAMMA-AMINOBUTYRIC ACID (GABA) AGONISTS. H. Bergen* and D.W. Pfaff. Rockefeller University, New York, NY 10021. (SPON: C. Mobbs)

GABA appears to exert an inhibitory effect on LHRH neurons in the preoptic area (POA) (Lamberts et al., Exp. Brain Res. 52:356,1983). GABA could act by (a) decreasing LHRH gene expression or LHRH synthesis, or (b) decreasing the electrical activity of the cell and the release of LHRH. We tested hypothesis (a) by exposing LHRH neurons to GABA agonists in vivo and observing whether changes occur as determined by LHRH immunocytochemistry. Mature female rats were ovariectomized and bilateral cannulae were implanted just dorsal to the POA. Ten days later an inner cannula, containing either THIP ($GABA_A$ agonist), baclofen ($GABA_B$ agonist), or neither (control), was inserted into each guide cannula. After 6 or 24 hours the rats were sacrificed, perfused and LHRH examined by immunocytochemistry. Neither THIP nor baclofen decreased the number of LHRH-ir cells observed, as compared to controls. In fact, there was a trend toward increased staining intensity of cells exposed to THIP (but not baclofen) as compared to controls. These results show that exposure of LHRH cells to GABA agonists does not directly reduce peptide levels, so that electrical activity decreases may comprise the primary mechanism of their neuroendocrine effect.

528.10

EXCITATORY AMINO ACIDS (EAA) STIMULATE LUTEINIZING HORMONE-RELEASING HORMONE (LHRH) SECRETION FROM ARCUATE NUCLEI-MEDIAN EMINENCE FRAGMENTS IN VITRO. A. Donoso*, F.J. Lopez*, and A. Negro-Vilar, Reproductive Neuroendoc. Sec., Lab. of Molec. and Integrat. Neurosci., NIEHS, Res. Tri. Park, NC 27709

We evaluated the role of EAA on LHRH secretion using agonists or antagonists acting through the different subtypes of glutamate receptors. Arcuate nuclei-median eminence (AN-ME) fragments from male rats were incubated in vitro in a Krebs-RingerBicarbonate (KRB) buffer and release of LHRH was measured by RIA. Glutamic acid (GLU) enhanced LHRH release from the nerve terminals at [10 mM]. The EAA agonists kainic (KA) and quisqualic (QA) acids at [1mM] evoked a 2-fold increase of LHRH release over basal levels. N-methyl-D-aspartic acid (NMDA) was inactive in a wide range of doses (from [0.05 mM] to [5 mM]). The threshold doses for the stimulatory effects of both KA and QA were decreased by increasing the K^+ concentration (14 mM) in KRB which induced a minor depolarization of the terminals. The range of minimal effective doses was KA [0.075 mM]>QA [0.1 mM]>GLU [10 mM]. NMDA was inactive in all the conditions tested, even in the presence of high K^+ or Mg^{2+}-free medium. The selective antagonist of KA/QA receptors, DNQX, blocked KA-and GLU-induced LHRH release, whereas the NMDA selective antagonist APH had no effect on KA and GLU responses. These findings suggest that EAA on stimulates LHRH release from AN-ME nerve terminals acting on non-NMDA glutamate receptor sites.

528.11

LUTEINIZING HORMONE RELEASING HORMONE RESPONSE *IN VITRO* TO γ-AMINOBUTYRIC ACID. G.A. Dissen, N.H. McArthur† and P.G. Harms*. Depts. An. Sci. and Vet. Anat.†, Texas A&M Univ., Coll. Stat., TX 77843-4271.

GABA has been proposed to be a regulator of LHRH release . Hypothalamic tissue, from adult male and ovariectomized (OVX; 3 to 4 weeks) rats, was placed in 12 x 75 mm polypropylene culture tubes containing 0.6 ml of Krebs-Ringer bicarbonate medium (10 mM glucose, 0.1% bovine serum albumin and 0.06 g/l bacitracin) and incubated for 2 hours at 37°C under an atmosphere of 95% O_2 and 5% CO_2 in a vortex mixer. At 30, 60, 90 and 120 min of incubation 0.5 ml of media was removed from each tube (samples 1-4 respectively) and replaced with fresh media. Release of LHRH (pg/30 min) into the incubate was determined by RIA and all values expressed are the difference between samples 2 and 3 (3-2; mean difference; MD). Generally, control tissues released less LHRH in the 3rd sample than the 2nd resulting in negative values. Tissues were treated during the 3rd sample (60-90 min) with GABA or GABA analogues. Male rat medial basal hypothalamus (MBH) with attached infundibulum (INF) released increased concentrations of LHRH when treated with 0.1 mM (5.9± 2.5/12; [MD pg/30 min]±SEM/n; p<.05) but not 1.0 mM (1.9±1.1/11) GABA, compared to control (C), -1.6±1.5/11. LHRH release from OVX rat tissue treated with GABA was not different from control. Tissues treated, mean differences and dosages administered were as follows: INF (C,-2.6±1.0/8), 0.1, 1.0 mM GABA; INF nucleus with attached INF (C, -0.01±0.1/8), 0.1,1.0 mM GABA; MBH with attached INF (C, -3.1±0.5/64), 0.004, 0.008, 0.01, 0.02, 0.04, 0.08, 0.4, 0.8, 1.0 mM GABA; and preoptic area with attached INF (C, 1.1±0.8/12), 0.1, 1.0 mM GABA. Also, the MBH from OVX rats (C, -3.1±0.5/64) did not respond to $GABA_A$ analogues muscimol (1.0 mM), bicuculline (0.01, 0.1 mM), nor $GABA_B$ analogues baclofen (1.0 mM), phaclofen (0.01, 0.1, 1.0 mM). Further, there was no difference from control MBH from OVX rats due to the combination of a $GABA_A$ agonist and $GABA_B$ antagonist (1 mM muscimol+1 mM phaclofen) nor the reverse (1 mM baclofen+0.1 mM bicuculline). From these *in vitro* data it appears GABA does not act to modify LHRH release within the hypothalamus or preoptic area of OVX rats as it does in male rats.

528.12

DOPAMINE REGULATION OF LHRH RELEASE: ULTRASTRUCTURAL AND IN VIVO ANALYSIS. C.D. Conover*, R.O. Kuljis and J.P. Advis* (SPON: L. Grandison). Dept Animal Sci, Rutgers Univ, New Brunswick, NJ 08903 and Dept Neurol, College of Med, Univ of Iowa, Iowa City, IA 52242.

We have recently reported the presence of synaptic contacts between dopamine (DA)- and LHRH-containing processes in ewe median eminence (ME, Kuljis and Advis, Endocrinology, 124:1579, 1989). This study further characterizes such interaction from a neuroanatomical and a physiological viewpoint. Tridimensional reconstructions of dopaminergic synapses onto LHRH processes, were analyzed using an immunocytochemical double labeling protocol described previously. In addition, we assessed in vivo ME release of LHRH in response to DA (100 µM) and its antagonist sulpiride (100 µM), perfused through a push-pull cannula (PPC). Perfusions and PPC sampling were performed at different antero-posterior levels of the ME, using a multiple guide cannula assembly. Tyrosine hydroxylase (TH)- positive presynaptic elements contained tightly packed round labeled synaptic vesicles and scattered labeled dense core vesicles. LHRH-positive postsynaptic elements were separated from the TH-containing terminals by a clearly identifiable synaptic cleft, which included an intervening dense band and a postsynaptic density characteristic of assymetric synapses. In vivo LHRH release from the posterior ME decreased during the perfusion of DA (20±3 vs 8±1 pg/200 µl perfusate, mean±sem, n=8) and increased during the perfusion of Sulpiride (18±4 vs 28±1 pg/200 µl perfusate, n=4). However, perfusion of DA in the anterior ME had no effect (n=4) or increased (n=5) in vivo LHRH release (21±4 vs 31±6 pg/200 µl perfusate). These results indicate that: **a)** synapse-mediated regulatory DA-LHRH interactions occur at the level of the ME and **b)** an inhibitory DA-LHRH interaction take place in the postero-lateral ME (USDA 87CRCR-1-2558).

528.13

DIURNAL MODULATION OF RAT HYPOTHALAMIC GONADOTROPIN-RELEASING HORMONE (GnRH) RELEASE BY MELATONIN IN VITRO. D.D. Rasmussen. Department of Reproductive Med., UCSD, La Jolla, CA 92093.

The mechanism by which melatonin exerts anti-gonadotropic activity remains unresolved. Accordingly, we used acute in vitro incubations of male rat hypothalamic tissues to investigate effects of melatonin (MEL) on hypothalamic GnRH secretion. At 1000 h (3.5 h after lights on) addition of 10^{-9} M, but not 10^{-7} or 10^{-5} M, MEL to the incubation media decreased ($p<0.05$) GnRH release from individual median eminences (n=15/trtmnt), whereas at 1500 h none of the MEL dosages significantly altered GnRH release (n=15/trtmnt). In contrast, at 1000 h 10^{-7} M MEL, but not 10^{-9} or 10^{-5} M MEL, increased ($p<0.05$) GnRH release from mediobasal hypothalami (MBHs, n=10/trtmnt), which included the arcuate nucleus. Results of the experiments with median eminences (suppression of GnRH release by MEL at 1000 h but not 1500 h) are consistent with evidence that MEL inhibits dopamine (DA) release in the rat hypothalamus early, but not late, in the photoperiod, and that DA increases GnRH release from the isolated median eminence. If the GnRH response to MEL is indeed mediated by suppression of DA release, results of the experiments with MBHs (stimulation of GnRH release by MEL) are likewise consistent with our recent demonstration (1989 Endo. Soc. Abst.) that although DA stimulates GnRH release from the median eminence in vitro, when the arcuate nucleus (containing beta-endorphin neuronal perikarya) is included and connections to the median eminence maintained, DA can inhibit GnRH release by an endogenous opiate mediated mechanism. We hypothesize that MEL inhibited GnRH release from the median eminence in a diurnal manner by inhibiting DA release in the a.m. but not p.m. When the arcuate nucleus was included, MEL-induced suppression of DA release resulted in decreased endogenous opiate activity, allowing increased GnRH release. (NIH HD-22608)

528.14

PHORBOL ESTER STIMULATION OF IN VITRO HYPOTHALAMIC GnRH RELEASE: EFFECT OF CHRONIC DIABETES MELLITUS. Thomas S. King and Inn Soo Kang*, Depts. of CSB and OB-GYN, University of Texas Health Science Center, San Antonio, TX 78284

Infertility is a common sequela to chronic diabetes in animal models as well as in humans. This has been attributed both to alterations in hypothalamo-pituitary and pituitary-gonadal activity. Commonly, serum LH and testosterone levels are decreased which undoubtedly account for the reduction in or absence of spermatogenesis in males. We sought to determine the effects of streptozotocin (STZ)-induced diabetes on hypothalamic gonadotropin releasing hormone (GnRH) release *in vitro*, relative to the effects of STZ-induced diabetes on circulating LH and FSH levels. Young adult male (Sprague-Dawley) rats were injected i.p. either with 65mg/kg of STZ or with vehicle only. Diabetics were hyperglycemic throughout the 8 month study period (456.8±51.5mg/dl in diabetics [n=6] vs. 118.9±21.8mg/dl in controls [n=6]). At the end of the study period, serum LH and FSH levels were decreased in diabetics vs. controls. Hypothalamic slices were perfused with a modified Krebs-Ringer buffer (pH 7.4) using a programmable perfusion system. Perfusion results are as follows: 5 min pulses of 60mM KCl increased GnRH release in control groups (n=6). In diabetic groups (n=6), basal GnRH release was somewhat less that that of controls; 60mM KCl induced GnRH release was attenuated in the diabetic group. Similarly, while 10 min pulses of phorbol 3,5-dibutyrate increased GnRH release in dose-response fashion (0.01-1µM) in control groups (n=6), response to this phorbol ester was greatly blunted in the diabetic groups (n=6). These results suggest that hypothalamic GnRH release is inhibited in chronic diabetes. Further, diabetes is associated with inhibition of phorbol ester stimulated GnRH release *in vitro*, suggesting that the GnRH regulating activity of protein kinase C has been altered in diabetics. (Supported by the American Diabetes Association and by NIH grant HD-10102 [Neuroendocrine Core]).

528.15

EFFECTS OF HISTAMINE ON LH RELEASE FROM DISPERSED RAT PITUITARIES. E. Rolfes* and L. Jennes (SPON: K. Sikora-VanMeter). Dept. Anat., Wright State Univ. Sch. Med., Dayton, OH 45435.

The role of histamine in the regulation of gonadotropin secretion was evaluated in an "in vitro" system of cultured pituitary gonadotropes. Pituitary glands of 50 day old female rats were dispersed with trypsin and cultured for 2 days in medium 199. Cells were then incubated for 3 hours in medium containing 1 pM to 10 uM histamine in the presence or absence of various GnRH analogs. Low concentrations of histamine (1pM) resulted in a 7-10 fold increase in LH release which declined in a dose-dependent manner with increasing concentrations of histamine. Thus, 10 pM of histamine caused only a 3-5 fold increase in LH release while in the presence of 1 nM histamine, the LH levels were close to baseline. Further increase of histamine had no effects on LH release. Coincubation with a GnRH antagonist blocked the LH stimulating effects of low doses of histamine. Estrogen decreased the histamine- and GnRH-stimulated LH release. The results suggest that histamine can act directly on pituitary gonadotropes to stimulate LH release and that the magnitude of the LH release is modified by estrogen.

528.16

EFFECTS OF NALOXONE ON FASTING INDUCED INHIBITION OF PULSATILE LH SECRETION. Dana L. Helmreich and J.L.Cameron. Depts. of Behavioral Neuroscience and Psychiatry, Univ. of Pittsburgh, Pittsburgh PA 15260

We have previously shown that one day of fasting can decrease the frequency of luteinizing hormone (LH) and testosterone (T) pulses in adult male monkeys, and that this slowing appears to occur at the level of the GnRH pulse generator. (Cameron and Nosbisch, Endocrine Society Abstract 200, 1989). To test whether this inhibition of LH secretion is due to an increased release of endogenous opioid peptides, we measured pulsatile LH and T secretion in chronically catheterized male rhesus monkeys (Macaca mulatta) during a 4hr saline infusion from 1200-1600 and a subsequent 8hr naloxone infusion (0.25 mg/kg·hr) from 1600 to 2400 on a day of normal eating, and again on a day of fasting. The results are summarized in the following table:

Hour	Saline 1200-1600	Naloxone 1600-2000	Naloxone 2000-2400
No. of LH pulses/4hrs			
Fed(n=6)	0.83±.28	3.67±.45*	1.83±.44
Fasted (n=6)	0.33±.19	1.33±.38ª	0.67±.38
Mean T (ng/ml)			
Fed(n=3)	1.26±.49	9.2±.76*	13.88±1.66*
Fasted(n=3)	0.67±.14	4.2±1.46	4.79±2.25ª

* significantly different ($p<.05$) from values during saline infusion
ª significantly different ($p<.05$) from values of fed group in same period

We conclude from these data that there is an endogenous opioid tone in intact male monkeys that tonically inhibits pulsatile LH release, and moreover, that endogenous opioid peptides are not responsible for fasting induced inhibition of LH secretion.

528.17

BRAIN SITES OF OPIOID INHIBITION OF LH SECRETION IN THE EWE. C.S. Whisnant* and R.L. Goodman (SPON: R. Millecchia) Department of Physiology, West Virginia University Health Sciences Center, Morgantown, WV 26506

This research sought to determine the sites of action where endogenous opioid peptides (EOP) inhibit luteinizing hormone (LH) secretion in the sheep brain. In the first experiment, ewes (n=4) were subjected to anterior deafferentation of the medial basal hypothalamus (MBH) with a Halasz knife. Cuts (180^0) were placed at the posterior border of the optic chiasm in ovariectomized, progesterone (P) implanted ewes in the anestrous season. Controls (n=6) received either sham cuts or no surgery. LH secretion was monitored in blood samples taken every 12 min for 3 h before and after i.v. administration of 12.5 mg of WIN 44,441-3 (WIN), an EOP antagonist. WIN increased LH secretion in both control and deafferentated ewes. In the second experiment, six ovary intact ewes were implanted with guide tubes in either the preoptic area (POA) or MBH during the breeding season. During the luteal phases of recurring estrous cycles, matched tubes containing WIN were placed through the guide tubes into these areas (2-4 times/site) and LH secretion was determined in blood samples taken as above. In some ewes, empty tubes were placed to serve as controls. Placement of tubes containing WIN in both the POA and MBH increased ($p < .01$) LH pulse frequency, but had no effect on LH pulse amplitude. These results suggest that EOP inhibit LH secretion at multiple sites in the ovine hypothalamus. Furthermore, although the EOP antagonist was effective when placed in the POA, input from the POA is not required since WIN (i.v.) increased LH secretion in anterior deafferentated ewes. Research supported by NIH HD 17864.

528.18

INTERACTION BETWEEN PENTOTHAL AND NALOXONE ON THE ACTIVITY OF THE HYPOTHALAMIC LHRH PULSE GENERATOR IN THE RHESUS MONKEY.
P.M. Grosser*, J-C. Thalabard*, C.L. Williams*, K.T.O'Byrne*, M. Nishihara*, and E. Knobil, Laboratory for Neuroendocrinology, University of Texas Health Science Center, Houston, TX 77225.

Rhythmic increments (volleys) of multiunit activity (MUA), synchronous with the pulsatile secretion of LH, can be recorded from the medial basal hypothalamus. Ovariectomy (OVX) prolongs volley duration 10-fold and estradiol (E_2) administration reverses this effect. Naloxone administration (1 mg/h) to ovary intact monkeys anesthetized by pentothal for unrelated reasons, increased volley duration from 4.7±0.5 to 8.8±0.7 min ($p < 0.001$; n=5 experiments), suggesting that endogenous opioids may be involved in mediating the effect of E_2. In later studies done in *unanesthetized* intact animals, however, naloxone had no effect (control 3.1±0.2 min; naloxone-treated 3.4±0.2 min; n=4 experiments). Furthermore, the reduction in volley duration by E_2 given to *unanesthetized* OVX animals was not prevented by naloxone (OVX 21.6±0.9 min; E_2-treated 12.9±0.7 min; E_2+naloxone 13.7±0.8 min; n=6 experiments). These results invalidate our original conclusion based on anesthetized animals and indicate an interaction between pentothal and naloxone in the functioning of this hypothalamic pulse generator. They also emphasize the need for caution in interpreting effects of opioid peptide inhibitors on neuroendocrine systems in the presence of other neuroactive drugs. (Supported by NIH grants HD 07324 and HD 17438 and by The Clayton Foundation for Research.)

528.19

EFFECTS OF NALOXONE ON LHRH RELEASE AS ESTIMATED BY INTRA-HYPOPHYSIAL MICRODIALYSIS. J.M. Meredith*, L.B. Rucker* and J.E. Levine (SPON: D. Ferster) Dept. of Neurobiology and Physiology, Northwestern University, Evanston, IL 60208.

Microdialysis was used to determine if pituitary LHRH levels are changed by treatment with naloxone (NAL), an opiate receptor antagonist that has been shown to stimulate LHRH release at hypothalamic sites (Karahalios and Levine, 1988). Our goals were to determine if LHRH responses to NAL measured by intra-hypophysial microdialysis resemble hypothalamic responses to NAL, and to assess opioid inhibitory tone in intact and castrate rats. To estimate LHRH levels, CMA/10 microdialysis probes (Carnegie Medicin) were selected on the basis of in vitro tests which confirmed suitable exchange rates and responsiveness to pulse-like changes in LHRH concentrations. During in vivo microdialysis (4.5ul/min flow rate) samples were collected every 5min for up to 11h. At 2, 5 and 8h, rats received NAL (2.5 or 10 mg/kg i.v) or saline. In 5 animals (3 castrate and 2 intact; 9 total trials), NAL increased mean LHRH release by an average of 32.7% ($p<.05$). In 7 non-dialyzed rats (4 castrate and 3 intact), 2.5mg/kg NAL increased mean plasma LH levels by an average of 22.1% ($p<.025$). We conclude that LHRH levels in hypophysial dialysates likely reflect ongoing LHRH neurosecretory rates, inasmuch as they are increased by NAL. Since NAL-induced increases in LHRH and LH were similar in intact vs castrate rats, these data are also consistent with previous reports suggesting that endogenous opioid peptides do not serially mediate gonadal negative feedback actions in rats.

528.20

ESTROGEN INDUCTION OF A LUTEINIZING HORMONE (LH) SURGE DURING THE LUTEAL PHASE OF THE RHESUS MONKEY: ROLE OF ENDOGENOUS OPIATES. D.A.VanVugt*, L.Heisler*, and M.Webb*. Depts. of Obstetrics & Gynecology and Physiology, Queen's University, Kingston, Ontario K7L 3N6. (Spon: R. Eikelboom).

Progesterone blocks the estrogen-induced LH surge in the monkey by an action on the hypothalamus. The objective of the present study was to determine if endogenous opiates are involved in progesterone inhibition of estrogen-induced positive feedback. Rhesus monkeys (macaca mulatta) were injected iv with nalmefene, a long acting opiate antagonist, or an equivalent volume of saline every 12h beginning 48h before and extending 72h after sc implantation of 17-B-estradiol containing silastic capsules. Blood samples were collected by venapuncture and serum LH concentrations determined by RIA. An LH surge occurred in 4 of 7 experiments in which nalmefene was given whereas no LH surge was observed when saline was substituted for the opiate antagonist. LH surges peaked 30-48h after sc insertion of the estradiol capsules. The mean amplitude of the LH surge was 96±18 ng/ml (range = 61-140 ng/ml), a significant increase from a mean basal concentration of 21±1.3 ng/ml. We conclude from these results that luteal phase monkeys responded to an estradiol challenge with an LH surge because nalmefene antagonized endogenous opiates. These results suggest that endogenous opioid inhibition of the hypothalamic pituitary axis may explain mechanistically why estrogen does not normally elicit an LH surge during the luteal phase. This work supported by a grant from the MRC of Canada.

ISCHEMIA: ENERGY METABOLISM AND ISCHEMIC MODELS

529.1

^{31}P NMR STUDIES OF RAT HIPPOCAMPAL SLICES AS A MODEL OF CEREBRAL ISCHEMIA

R.K. Gupta*, F.A.X. Schanne* and P.K. Stanton. Albert Einstein College of Medicine, Bronx, NY 10461.

Intracellular high energy phosphates (HEP) were monitored in rat hippocampal slices by ^{31}P NMR under conditions of continuous perfusion, no flow and reperfusion, to model changes which occur during cerebral ischemia and reperfusion injury.

Hippocampal slices (350-400 μm thick) were prepared from adult male Sprague-Dawley rats (100-200 g; 6-7 per experiment) using a Vibratome slicer by standard methods. Slices (60-70) were placed on nylon nets in a 10 mm diameter NMR tube and perfused at 20 ml/min with artificial cerebrospinal fluid (composition (mM): NaCl 126, KCl 5, NaH_2PO_4 1.25, $MgCl_2$ 2, $CaCl_2$ 2, $NaHCO_3$ 26, glucose 10). Slices were maintained at 30° C and perfusion media saturated with 95% O_2/5% CO_2 (pH 7.4). ^{31}P NMR spectra were acquired on the Varian VXR 500 spectrometer with the 10 mm probe tuned to 202.3 MHz for phosphorous-31. (NMR parameters see previous abstract).

Hippocampal slices, under these perfusion conditions, exhibited stable (>2 hrs) intracellular HEP resonance signals. Interrupting perfusion produced decreases in pH and phosphocreatine levels (10% of control levels after 15 min) far more rapidly than in isolated cells, followed by losses in nucleotide phosphates. A shift in the inorganic phosphate resonance frequency indicated lowered pH during no flow. With reperfusion, pH recovered and HEP returned towards normal levels, depending upon the duration of no flow. The more rapid time course of HEP reductions suggests factors in the slice (such as synaptic activity) may make it a useful ischemia model containing an integrated functional network of neurons and glia more closely paralleling the *in vivo* situation.

529.2

^{31}P NMR STUDIES OF NG108-15 CELLS AS A MODEL OF CEREBRAL ISCHEMIA

F.A.X. Schanne*, J.R. Moskal, and R.K. Gupta* (SPON: D. Spitzer). Albert Einstein College of Medicine, Bronx, NY 10461.

Intracellular high energy phosphates (HEP) were monitored in the neuroblastoma x glioma cell, NG108-15, by ^{31}P NMR under conditions of continuous perfusion, no flow and reperfusion to model changes occuring during cerebral ischemia and reperfusion injury.

NG108-15 cells were attached and grown on Cytodex microcarrier beads in Dulbecco's modified Eagle's medium supplemented with 5% fetal bovine serum in 60 mm petri dishes at 37° C. Cells on beads were transferred to a 10 mm diameter NMR tube and perfused with culture medium at a flow rate of 2 ml/min. Cells were maintained at 30° C and the perfusion medium saturated with 95% O_2/5% CO_2 (pH 7.4). ^{31}P NMR spectra were obtained on a Varian VXR 500 spectrometer using a 10 mm probe tuned to 202.3 MHz for phosphorous-31. NMR parameters used were: acquisition time, 0.8 sec; pulse angle, 90°; spectral width, 20 KHz; and a recycle time of 1.6 sec.

Under these perfusion conditions, stable intracellular HEP resonance signals were observed. Upon stopping the perfusion, there were rapid decreases in pH and a slower fall in phosphocreatine (10% of control levels after 45 min), followed by losses in nucleotide phosphates. Upon reperfusion, the pH returned to normal and the HEP recovered towards normal levels, depending upon the duration of no flow. These pattern of changes is similar to that observed by ^{31}P NMR of cerebral ischemia *in vivo*. This *in vitro* model of cerebral ischemia in a single neuronal cell line may prove useful in elucidating mechanisms of ischemic and reperfusion injury, and provide a test system for studying protective agents.

529.3

DISTRIBUTION OF CREATINE KINASE ACTIVITY IN GERBIL HIPPOCAMPUS BEFORE AND AFTER ISCHEMIA. T.S. Whittingham, J. Sheer*, H. Assaf* and W.D. Lust. Dept. of Oral Biology and Div. Neurosurgery, CWRU Schools of Dentistry and Medicine, Cleveland, OH 44106.

Previous studies have shown that neural function is maintained for longer periods of anoxia when phosphocreatine is increased prior to the insult. This implies that creatine kinase (CK) is located in critical neuronal regions. Tests are currently being performed to determine the distribution pattern of CK in the gerbil hippocampus. Adult male mongolian gerbils are grouped as follows: Control (no ischemia) and four post-ischemic groups (2,3,4 and 10 days reflow after 5 min bilateral common carotid artery occlusion). The post-synaptic neural elements are gradually lost in area CA1 during the reflow period, allowing separation of CK activity from pre- and post-synaptic elements. Twenty-micron frozen brain sections are prepared and lyophilized, and 1 μg samples are dissected from fiber tracts, synaptic regions and cell body layers in the dentate gyrus and areas CA1 and CA3. Tissue samples are assayed for CK activity by standard histochemical fluorometric methods.

529.4

SYNAPTOSOMAL RESPIRATION DURING NEUROLOGICAL DETERIORATION FOLLOWING ANOXIA. K.R. Wagner, M. Kleinholz* and R.E. Myers. Research Service, VAMC; Dept. of Neurology, Univ. Cincinnati Coll. Med., Cincinnati, OH 45220.

Hyperglycemic but not normoglycemic animals exposed to anoxia develop a delayed appearance of neurologic signs including fasciculations, focal and tonic-clonic seizures and coma during the hours following reoxygenation. These signs appear after a symptom-free period. Biochemical studies demonstrate marked inhibition (50-60%) of ADP- and uncoupler-stimulated respiration by isolated non-synaptic ('free') brain mitochondria from cats sacrificed with neurologic signs (J. Neurochem., 1989). These respiratory impairments lead to 5 to 10-fold reaccumulations of lactic acid and reductions in ATP (30%) and phosphocreatine (50%) concentrations in postanoxic brain tissue. Asymptomatic cats fail to show mitochondrial or metabolic alterations.

Presently, we measured oxygen consumption rates by synaptosomes isolated from symptomatic and asymptomatic cats. Similar degrees of respiratory stimulation were present with either veratridine or an uncoupler in synaptosomes from symptomatic and asymptomatic animals. Non-synaptic mitochondrial function was impaired only in symptomatic cats. These results suggest that either synaptic mitochondria are resistant to postanoxic injury or that mechanisms damaging non-synaptic mitochondria are not present at synpases during the development of neurological deterioration following hyperglycemic anoxia.

529.5

EFFECTS OF ANOXIA AND AGLYCEMIA ON A THICK HIPPOCAMPAL SLICE MODEL OF ISCHEMIA. G.C.Newman, F.E.Hospod, T.S.Whittingham. Dept. of Neurology, SUNY at Stony Brook, Stony Brook, NY, 11794 and Dept. Oral Biology, Case Western Reserve, Cleveland, OH 44106. (SPON: J. HALPERIN)

We have previously demonstrated that 1000μ thick brain slices show increased brain slice glucose utilization (SGU), lactate, and inulin exclusion spaces but retain relatively normal histology and ultrastructure, even at the slice center. (Newman *et al*, JCBFM, 8:586,1988) In this study, we attempt to separate the effects of acidosis and energy depletion by studying four groups of slices subjected to 15 min insults: 1) CONTROL (pO_2 715, 4 mM glucose); 2) ANOXIC; 3) AGLYCEMIC; 4) ANOXIC-AGLYCEMIC.

Hippocampal brain slices were prepared from 175g Sprague-Dawley rats and pre-incubated for 90 min in K-R (1.5 mM Ca^{++}) at 37°C. Slices were then transferred to another chamber pre-equilibrated with one of the three deprivational buffers for 15 min or removed and returned to the same chamber for CONTROLS. Following return to well-oxygenated, 4mM glucose K-R, slices were removed for measurement of brain slice glucose utilization (SGU, see Newman *et al*, this meeting) after 5 min, 1 hour or 4 hours. SGU was quantified in eight regions of each slice.

SGU increases significantly in stratum radiatum (sRAD) of CA1 and CA3 compared to standard 540μ hippocampal slices. In CONTROLS, sRAD SGU declines by about 40% over 4 hours, consistent with our suggestion that the 1000μ brain slice models the ischemic penumbra. Slices exposed to AGLYCEMIA or ANOXIA-AGLYCEMIA show a decline of 75% over 4 hours whereas ANOXIC slices show a decline of 85%. We also observe a dramatic and transient increase in SGU in CA1 and CA3 stratum oriens immediately after ANOXIA or ANOXIA-AGLYCEMIA but not after AGLYCEMIA. We conclude that it should be possible to separate effects of acidosis from those of energy depletion without acidosis in this model system.

529.6

DIFFERENCES IN ANOXIC AND ISCHEMIC STATES OF UNDIFFERENTIATED N1E-115 NEUROBLASTOMA CELLS IN HIGH DENSITY CULTURES AS STUDIED BY ^{31}P NMR. P. Glynn[1]*, S. Ogawa[2], and R.L. Chappell[1] (SPON: K.M. Lyser) [1]Hunter College, 695 Park Ave., NY, NY 10021 and [2]Bell Labs, 600 Mountain Ave., Murray Hill, NJ 07974.

The effect of low oxygen was studied on N1E-115 neuroblastoma cells in a high density superfused microcarrier based tissue culture system. The results were compared with those obtained from cells subjected to ischemic episodes of 10 through 55 minutes. The object was to assess the effect of the anoxic condition on otherwise unstressed cells, and to infer the anoxic contribution to an ischemic episode. In the anoxic condition, the pH, Pi, and ATP levels remained constant for five hours while the phosphocreatine level dropped 48%. In contrast cells subjected to an ischemic response showed a rapid decline in pH and in phosphocreatine and ATP concentration. Recovery was directly proportional to the time of the ischemic condition. The percentage of cells killed was proportional to the length of the ischemic episode. For a 10 minute episode no cells were lost, for 55 minutes 40% of the cells were lost. These results show that an anoxic condition lasting several hours does not have a strong adverse effect on the metabolic state or survival of the cells and that an anoxic contribution to the ischemic condition exists which is capable of draining off approximately 3% of the high energy phosphocreatine per 10 minutes of ischemic event.

529.7

IN VIVO MICRODIALYSIS PERMITS DYNAMIC MONITORING OF METABOLITE CHANGES RESULTING FROM SPINAL CORD ISCHEMIA. K.E. Peek, C.S. Robertson*, A.H. Priessman* and J.C. Goodman. Dept. Neurosurgery, Baylor College of Medicine, Houston, Texas 77030.

Dynamic changes in lactate concentration in the spinal cord of rabbits were monitored before, during, and after occlusion of the abdominal aorta. Recordings of somatosensory evoked potential (SSEP) permitted concurrent monitoring of neuronal function. A loop of dialysis tubing (MWCO=6000) was inserted at L5 into the dorsal spinal cord of anesthetized animals. Dialysate samples were analyzed utilizing computer-assisted HPLC. Predictions of functional recovery were based on percent return of postsynaptic wave amplitudes.

In untreated animals, lactate levels peaked both during ischemia and again at 15 min reperfusion, with the latter peak being greater. Treatment with dichloroacetate (25 mg/kg) just prior to ischemia reduced the reperfusion peak of lactate; concomitant SSEP recordings indicated improved functional recovery. These results suggest that inhibition of pyruvate dehydrogenase kinase may facilitate a more rapid return to aerobic energy production, thus improving outcome.

529.8

POSTHYPOXIC GLUCOSE SUPPLEMENT REDUCES HYPOXIC-ISCHEMIC BRAIN DAMAGE IN THE NEONATAL RAT. H. Hattori*, C.G. Wasterlain. (SPON: M.K. Menon) Epilepsy Res. Lab., VAMC, Sepulveda, CA 91343; Dept. of Neurol., UCLA Sch. of Med., Los Angeles, CA 90024.

We evaluated the effect of posthypoxic glucose supplement in a neonatal hypoxic-ischemic animal model. Seven-day-old rats underwent bilateral ligation of the carotid arteries followed by exposure to 8% oxygen atmosphere for 1 hr. The extent of hypoxic-ischemic brain damage was assessed histologically 72 hr later. Supplement of 10% body weight of 5.4% glucose immediately after the hypoxic exposure reduced the volume of neocortical infarction to 37% of the control value, and attenuated ischemic damage in striatum and dentate gyrus. At the end of the hypoxic exposure, glucose was 7 mM in plasma and 0.3 mmol/kg in brain. Plasma and brain lactate were 10 mM and 9 mmol/kg respectively. Glucose supplement produced a rapid rise of glucose in plasma to 25-30 mM and in brain to 3-5 mmol/kg over the next 2 hr. Lactate in both plasma and brain gradually fell toward the baseline level during the first hour of recovery regardless of posthypoxic glucose supplement. In this model, restoring brain glucose after the insult reduced hypoxic-ischemic infarction and did not raise brain lactate to toxic levels. These results illustrate the important role of glucose in neonatal hypoxia-ischemia and the fact that full cortical infarction can develop independently of lactic acid accumulation.

529.9

RELATIONSHIP BETWEEN PLASMA GLUCOSE (PG) AND INFARCT VOLUME FOLLOWING FOCAL CEREBRAL ISCHEMIA REPERFUSION. He Y.Y.*, Yip P.K.*, Garg N*, Hogan E.L. and Hsu C.Y. Dept. of Neurology, Medical University of South Carolina, 171 Ashley Avenue, Charleston, SC 29425, USA

We studied the influence of PG on ischemic brain injury and the timing of this effect. Reperfusion following temporary occlusion of the right middle cerebral artery and both common carotid arteries results in a cortical infarct. The infarct volume (IV) 24 hr after ischemia is dependent upon the duration of the 3-vessel occlusion. In the fed rats, PG and IV were greater than those of rats fasted for 24 hr or fed rats treated with insulin (IN) (1.7 units/kg, IP 70 min before ischemia) (Table). Fasted rats receiving glucose during ischemia, (intra-ischemic PG 349±44) resulted in greater IV (118.5±67) than those with glucose loading immediately after ischemia (intra-ischemic PG 134±36; IV 18±15) or 3 hr post-ischemia (intra-ischemic PG: 137±35; IV 31±54). Results suggest that PG during ischemia, but not post-ischemic reperfusion has a significant effect on ischemic brain injury.

	PG	Infarct Volume (mm^3)			
	(mg/dl)	30 min	45 min	90 min	120 min
Fed	333±31	7±18	145±38	181±67	188±33
Fasted	115±18*	1± 2	5± 5*	149±46	148±64
Fed + IN	70±18*	-	-	59±62*	126±54*

Mean ± SD, n≥9, *denotes statistical significance from fed rats by t test ($p<0.05$). "-" denotes exp. not done.

529.10

GLYCEMIA LEVEL FOLLOWING CEREBROVASCULAR OCCLUSION IN CATS: EFFECT ON OUTCOME. M. Kleinholz*, G.M. de Courten-Myers*, R.E. Myers, K.R. Wagner (SPON: B. Liwnicz). Research Serv. VAMC; Dept. Pathol. UC Coll. Med., Cincinnati, OH 45220.

Hyperglycemia adversely affects infarct size from middle cerebral artery occlusion (MCA-O)(Stroke 19:623, 1988). Presently we investigated a) normalizing hyperglycemia after occlusion by replacing glucose (Glu) with saline (Sal) infusions with and without insulin (Ins) and b) elevating serum glucose concentrations postocclusion in pentobarbital anesthetized cats. Infarct size measured after 2 weeks survival is % of MCA territory. Acute deaths resulted from MCA territory edema with maximal infarcts.

Infusion Pre/Post		Glycemia (mM) Through Time					Edema Deaths	Infarct Size
MCA-O	N	MCA-O	+1	+2	+6	+8H	(%N)	(Mean+SE)
Glu/Glu	12	20	20	20	20	10	16.7	41+ 9
Glu/Ins	8	20	5	3-4	3-4	8	37.5	36+10
Glu/Sal	13	20	12	8	7	8	30.8	23+ 6
Sal/Glu	13	8	15	20	20	10	15.4	18+ 5
Sal/Sal	13	8	8	8	9	10	0.0	14+ 4

Conclusions: Neither slow nor rapid reductions of hyperglycemia following MCA occlusion with saline or insulin infusions, respectively, improved overall outcome. However, survivors that received postocclusion saline trended towards smaller infarcts versus those receiving glucose. Hyperglycemia after normoglycemic MCA occlusion yielded a worse outcome than continued normoglycemia ($p<0.05$).

529.11

2-DEOXYGLUCOSE STUDY OF THE CONSEQUENCES OF TRANSIENT BRAIN ISCHEMIA IN GERBILS AND THE PROTECTIVE EFFECTS OF MK-801. C.M.Tomaski* and B.E.Morton (SPON: J Hardman). Dept Biochem and Biophys, Univ. Hawaii Sch. of Med., Honolulu, HI 96822.

Transient brain ischemia causes release of glutamic acid in large amounts. The glutamate binds NMDA receptors to open neuronal calcium channels. After 24 hours, cell death occurs, possibly due to ATP depletion from calcium pumping. This chain of excitotoxic events has been interrupted with NMDA receptor antagonists or by blocking the linked calcium channel with "indirect" NMDA antagonists, such as MK-801.

Bilateral ischemia was produced in gerbils under halothane anesthesia by clamping the common carotid arteries for 5 minutes. 125 µCi/kg 2-deoxy-D-glucose-1-14C was injected, i.p., at 1 minute pre-, and up to 7 days post-clamping. After 45 minutes, brains were removed and frozen. Autoradiograms of 20 µm sections were prepared and film densities were quantitated at 30 brain sites.

In ischemic animals, hippocampal and caudate glucose uptakes were initially elevated. By 4 days these were markedly depressed, with Nissl stain showing necrosis. MK-801 (3 mg/kg, i.p.) given 1 hour post-clamping, did not prevent the early increases in regional glucose uptake. However, MK-801 prevented essentially all later reductions in glucose uptake and necrosis. These results support the view that the early increases in glucose metabolism due to ischemia are not sufficient to cause later neuronal death. Supported by American Heart Association Grant A-3423.

529.12

DELAYED REPETITIVE CEREBRAL ISCHEMIA: BEHAVIORAL AND NEUROPATHOLOGICAL OBSERVATIONS. D. Wang*, D. Corbett and S. Evans* (Spon: D.W. McKay). Faculty of Medicine, Memorial University, St. John's, NF, Canada, A1B 3V6.

Repetitive cerebral ischemic episodes are most commonly found in Transient Ischemic Attack (TIA) and stroke patients. At present no animal model involving delayed repetitive cerebral ischemia has been used to study functional deficits.

Cerebral ischemia was induced in gerbils by a 10 min bilateral occlusion of the carotid arteries using a chronically implanted occluding device. The occluding procedure was performed once a week for 3 weeks. The large increase in locomotor activity that resulted from the first occlusion gradually subsided over subsequent test days. The second and third occlusions failed to further alter locomotor activity. Animals experiencing one ischemic episode exhibited marked cell loss within CA 1 of the hippocampus whereas damage in CA 2 and CA 3 was noted after multiple ischemic episodes.

These data indicate that the increase in locomotor activity was correlated with the initial damage to CA 1 neurons. Remaining undamaged neurons may have permitted "spatial maps" to be formed such that locomotor activity declined over test days. This model may aid in the development of drugs for treatment of TIA and stroke patients.

529.13

LONG TERM FUNCTIONAL IMPAIRMENTS FOLLOWING CEREBRAL ISCHEMIA IN THE GERBIL. D. Corbett and D. Wang*. Faculty of Medicine, Memorial University, St. John's, NF, Canada, A1B 3V6.

A brief period of cerebral ischemia produces a marked increase in locomotor activity which may be due to the ischemic animal's inability to form a "spatial map" of the test environment. If this idea is correct, the increase in locomotor activity should be a relatively permanent change since ischemia causes severe damage of the hippocampus.

Eighteen anesthetized gerbils were subjected to either bilateral carotid artery occlusion for a period of 5 min (N=8) or sham surgery (N=10). The gerbils were tested in an open-field beginning 24 hrs after surgery and continuing each day for 10 days.

The ischemic animals exhibited a large increase in locomotor activity compared to control animals ($p<.001$, ANOVA). The level of locomotor activity declined over the 10 day test period but remained twice that of control animals. The CA 1 region of hippocampus was extensively damaged in ischemic animals.

A brief period of ischemia produces a long lasting deficit in spatial mapping ability. The limited recovery that takes place may be mediated by other brain regions involved in spatial mapping (e.g. prefrontal cortex).

529.14

SOMATOSENSORY EVOKED POTENTIALS (SEP) AND NEURONAL DEGENERATION AFTER MIDDLE CEREBRAL ARTERY OCCLUSION IN RATS. K. Sakatani, H. Iizuka, W. Young. Depts. of Neurosurgery, Physiology & Biophysics, NYU Medical Center, 550 First Ave., New York, NY 10016.

Middle cerebral artery occlusion (MCAo) produces infarcts in the rat frontoparietal cortex. We took advantage of the orderly somatotopic arrangement of cortical motor-sensory functions to study the neurophysiological and morphological consequences of MCAo. Vibrissae (trigeminal), median and sciatic nerve stimulation produced robust responses localized to the lateral parietal (Par1), forelimb (FL) and hindlimb (HL) cortical regions. MCAo abolished cortical responses in all 3 regions within minutes and dimished subcortical white matter responses. Par1 SEPs did not recover in any rat while FL SEPs recovered within an hour. HL SEP usually recovered within 30 minutes and exceeded preinjury levels by an hour. Rats with extensive infarcts did not recover SEP in all 3 regions. We used the Fink-Heimer (FH) method to study degenerating axons in the brainstem and spinal cord of the rats. MCAo caused unilateral degeneration of pyramidal tract. The distal extent of corticospinal tract degeneration reflected infarct size. All rats had marked degeneration of corticofugal axons to the trigeminal nucleus. FH staining revealed distinctive argyrophilic neurons by 6 hours after MCAo, present in all cortical layers close to the infarct but localized to upper cortical layers II-III at 0.5-2 mm from the infarct. By 3-7 days, massive terminal degeneration appeared in peri-infarct cortex but neither the number nor distribution of argyrophilic neurons progessed. Thus, the MCAo infarct always involved Par1, encroached upon FL, and seldom affected HL regions. MCAo caused widespread functional disruption and non-progressive neuronal loss in peri-infarct regions. The peri-infarct neuronal loss and SEP changes may result from spreading depression emanating from ischemic cortical regions.

529.15

SINGLE VESSEL RAT FOREBRAIN PERFUSION: ONE TECHNIQUE TO STUDY ACUTE ISCHEMIC AND/OR TRAUMATIC BRAIN INJURY. J. Zinkel* (SPON: P. Knapp). Department of Neurosurgery, Wayne State University School of Medicine, Detroit, MI 48201.

In the rat, forebrain perfusion can be reduced to that by one internal carotid artery alone without neurological deficit. This is done (for example) by occluding the left external carotid, right internal carotid, the distal basilar, both ptyergopalatine, and both occipital arteries.

Total hemisphere ischemia is studied in the awake rat by reversible occlusion (with a vessel loop) of the left common carotid artery. Partial ischemia is studied when catheters are placed retrograde into the left external carotid and left femoral arteries. The left common carotid artery is occluded and blood is pumped at a controlled rate from the femoral artery catheter through a perfusion pump and then into the hemispheres by way of the external carotid catheter.

To study closed head (diffuse axonal) injury the carotid loop is tightened in the awake rat. He lapses into decerebrate coma and the injury is then introduced. The carotid loop is loosened and hemisphere flow is restored (Note that the pharmacologic anesthesia is neither as deep nor as quickly reversible as is decerebrate coma as described.)

All surgical manipulations as described are easily tolerated by the rat. Cerebral injuries are introduced the day following surgery with the rat in decerebrate coma. Regional perfusion, histologic and neurologic outcome studies will be presented.

529.16

DEVELOPMENT OF A REPERFUSIBLE MODEL OF PHOTOCHEMICALLY INDUCED THROMBOTIC STROKE IN THE RAT MIDDLE CEREBRAL ARTERY (MCA) TERRITORY. B.D. Watson, R. Prado, A. Marrugo, Dept. of Neurology, Univ. Miami School Medicine, Miami, FL 33101

A reproducible and reversible model of stroke induced by occlusive arterial thrombosis in situ has not been reported previously. Occlusion of the rat MCA is easily done by irradiation with an argon laser beam interacting with intravenous photosensitizing dye. With rose bengal, a pure Type II (singlet oxygen) generator and 514.5 nm irradiation, the occlusion is composed entirely of agglutinated platelets; lack of fibrin content precludes recanalization with tissue plasminogen activator (rt-PA). With flavin mononucleotide (FMN), presumed to be a Type I (free radical) generator and 457.9 nm irradiation an unstable red clot forms; fibrin content is implied by negative Factor VIII and fibrinogen immunofluorescence. To enhance the free radical pathway, norbixin, a singlet oxygen (but not superoxide radical) quencher, was injected to a blood concentration of 13.4 uM, and followed by FMN at 1.34 mM. The MCA at the level of the olfactory tract was exposed and irradiated with 4 mW of 457.9 nm light dispersed longitudinally by a Ronchi ruling into 3 beams. Occlusion occurred within 10 min, yielding 3 colorless clots stable for 1 hour against saline injection. Upon bolus intracarotid injection of 200 ug rt-PA (n = 3), recanalization within 10 min was observed, which was stable for times up to 1 hr. This model may find utility in controlled studies of reperfusion injury.

529.17

CEREBRAL BLOOD FLOW DURING REPERFUSION AFTER FOCAL ISCHEMIA. W.R.Selman*, R.C.Crumrine*, K.A.Seta*, R.A.Ratcheson, and W.D.Lust. Lab. of Experimental Neurological Surgery, Case Western Reserve Univ. School of Medicine, Cleveland, OH 44106

Alterations in blood flow during early stages of reflow may be an important factor in the evolution of brain damage following focal ischemia. Spontaneously hypertensive rats (SHR) were anesthetized and the left middle cerebral artery occluded with a snare ligature for 1, 2 or 4 h. Cerebral blood flow (CBF) was determined by the ^{14}C-iodoantipyrine technique 5 min after clip removal. The mean CBF in the contralateral cortex from 10 SHR was 159 $\pm$ 13 ml/100g/min. Marked decreases in CBF were evident in both the striatum and cortex medial to the ischemic focus. CBF in the cortex was 48, 41 and 63% of control after 1, 2, and 4 h of ischemia, respectively. In contrast, CBF in the ischemic region was only slightly elevated (i.e., 110% of cont) during reflow. In both the perifocal and focal cerebral cortex, the changes in CBF appeared to be independent of the ischemic duration. Although the hyperemia observed during reflow after global ischemia was absent, it would appear that reperfusion triggers a shunting of CBF from the perifocal region to the affected area following focal ischemia.

529.18

IMPEDANCE IMAGING OF STROKE : PRELIMINARY ASSESSMENT IN A RAT MODEL. D.S. Holder* (SPON: L. Bindman). Dept. of Physiol., University College London, London WC1E 6BT, U.K.

The feasibility of impedance imaging (Applied potential tomography, APT) of cerebral ischaemia with epicortical or scalp electrodes has been assessed in an animal model. Cerebral ischaemia was produced in anaesthetized rats by diathermy of the vertebral arteries and reversible occlusion of the internal or common carotid arteries for 5 min. Impedance was measured at 50 kHz by a four electrode method with Ag/AgCl discs on the cortex or wires in the scalp, spaced 2mm apart. Impedance increased during occlusion by 25.2$\pm$14.0% on the cortex (mean $\pm$ S.D., n=22 in 10 rats) and 2.7$\pm$1.2% in the scalp (n=23 in 12 rats). Similar changes were obtained with wider electrode spacings. The scalp increases were due in part to cooling of 0.9$\pm$0.8^{o}C in recordings at ambient temperature, but increases of 1.4$\pm$0.7% were still seen when the scalp temperature increased by 0.9$\pm$1.1^{o}C (n=6 in 4 rats) due to external warming. Similar increases (3.9%$\pm$1.9%, n=12 in 4 rats) occurred when the scalp was surgically excised and then replaced before recording. These changes exceed the sensitivity of 0.1% impedance change of a prototype APT device (Brown, B.H., Barber, D.C. & Seagar, A.D., Clin. Phys. Physiol. Meas., 6:109-121, 1985). The scalp changes do not appear to be due to changes in temperature or other local scalp conditions. This suggests that APT could generate images of stroke, and work is in progress to assess its accuracy in this model.

529.19

EXTRAVASATION OF ALBUMIN IN THE HIPPOCAMPUS OF GERBIL BRAIN FOLLOWING TRANSIENT ISCHEMIA: LIGHT AND ELECTRON MICROSCOPIC IMMUNOCYTOCHEMICAL INVESTIGATION. M. Maeda*, F. Akai* and T. Yanagihara. Dept. Neurol., Mayo Clinic, Rochester, MN 55905 (SPON: D. W. Klass)

Breakdown of blood-brain barrier occurs following cerebral ischemia but the exact time sequence and parenchymal distribution of extravasated serum components are not certain. We therefore studied the distribution of serum albumin in gerbil brains after unilateral carotid occlusion for 10 min and subsequent reperfusion using light and electron microscopic immunocytochemistry. By light microscopy, no reaction for albumin was observed until 6 hrs after reperfusion and it was still weak and limited to the neuropil and a few neurons in the subiculum-CA1 region. A consistent reaction for albumin was not detected in the neuronal perikarya or neuropil in the subiculum-CA1 and medial CA1 region until reperfusion for 24 hrs and an intense reaction not until 48 hrs. Electron microscopic study of these areas revealed electron-dense precipitates in the extracellular space 30 min after reperfusion and in the dendrites and astrocytic cytoplasm after 1 hr. Electron-dense precipitates were observed in the degenerated neuronal perikarya after reperfusion for 24 hrs. Our study suggested that albumin leaked into the brain parenchyma promptly after re-establishment of reperfusion and accumulated not only in the astrocytes but also in the degenerated neuronal perikarya and dendrites.

529.20

MATHEMATICAL MODELING OF HYDROGEN CLEARANCE BLOOD FLOW MEASUREMENTS IN PERIPHERAL NERVE. T.D. Lagerlund and P.A. Low, Neurology Department, Mayo Clinic, Rochester, MN 55905

The hydrogen clearance method of blood flow measurement often yeilds biexponential wash-out curves. In peripheral nerve, which does not contain two discrete anatomical compartments, it has been suggested that arterio-venous shunt vessels may clear hydrogen, leading to the fast component of a biexponential curve. To assess this hypothesis, we simulated the wash-out of hydrogen from nerve tissue in the vicinity of a shunt vessel by modeling diffusion of hydrogen to the vessel and its transport by blood, in addition to removal of hydrogen by capillaries in the tissue surrounding the vessel. We calculated the hydrogen tension in each compartment as a function of radial distance from the vessel, axial distance along the vessel from arterial to venous end, and time. This was done for various values of parameters appropriate for rat sciatic nerves, and the resulting clearance curves were fit to a biexponential curve to determine the fast and slow clearance rates and the relative weights of the two components. The results show that the clearance rates and weights are affected by all model parameters including arterial clearance rate, capillary flow, blood velocity in the shunt vessel, diameter and length of the vessel, and distance between vessels. Thus, it is not easy to separate the contributions of arterial flow, shunt flow, and capillary flow to the clearance rates and weights.

529.21

EFFECT OF ISCHEMIA AND REPERFUSION IN VIVO ON ENERGY METABOLISM OF RAT SCIATIC NERVE. P.J. Zollman*, K.K. Ward*, J.D. Schmelzer*, and P.A. Low (SPON: J. Cunningham). Neurophysiology Laboratory, Department of Neurology, Mayo Foundation, Rochester, MN 55905

Our model of severe ischemic neuropathy consistently results in extinction of the nerve impulse within 30 min and reperfusion accentuates the reduction in nerve blood flow and blood-nerve barrier disruption.[1] Since impulse transmission may depend on nerve energy metabolism (NEM), we studied the effects of ischemia with reperfusion on NEM in vivo, in vitro, and postmortem. Ischemia for 30 min postmortem or in Ringer's solution resulted in marked depletion of adenosine triphosphate (ATP) and creatine phosphate (CP) and an increase of lactate in sciatic nerve from adult male Sprague-Dawley rats. In contrast, ischemia for up to 3 h in vivo did not deplete ATP, CP, or glucose and did not increase lactate. Ischemia with reperfusion times of 3 d to 3 weeks did not deplete any of the metabolites. The fact that energy metabolism remains intact in vivo under conditions which consistently results in complete abolition of the nerve impulse, suggest that nerve impulse extinction in vivo cannot be explained on the basis of energy substrate depletion in mammalian nerve. Presumably, the minimal residual perfusion is adequate to maintain energy stores, but inadequate to maintain impulse transmission.

1. Schmelzer JD, Zochodne DW, Low PA. Proc. Natl. Acad. Sci. 86:1639-1642, 1989.

529.22

A POSSIBLE NEUROPATHOLOGICAL MODEL FOR FETAL HYPOXIA

Y. Shen, F. Held*, W.P. Smotherman and R. L. Isaacson, Department of Psychology and Center for Developmental Psychobiology, SUNY Binghamton, New York 13901.

Using a method developed by Smotherman and his associates for the study of preterm rat fetuses without discomfort to the mother, we undertook to evaluate the effects of transient umbilical cord compression on oxidative metabolism as indicated by cytochrome oxidase histochemistry and the later development of behavioral capacities. The cord was compressed for 0, 2, 6, or 12 min in different groups of fetuses at E21. Half of the subjects were sacrified immediately after the clamping for histochemical analysis. The other half were delivered by cesarian section and cared for by a newly parturient female. The pups were tested at P3 for righting reflexes and for the ability to avoid falling from an elevated platform. The clamping of the cord produced a reduction in cytochrome reaction product that varied with the duration of the clamping. Regional differences in the amount of change in the cytochrome reaction product were noted. Behavioral consequences of the intervention included longer latencies for the righting response and poor performance on the elevated platform. These results suggest that this model may be useful for evaluating the neural and behavioral consequences of umbilical cord obstruction before birth.

WPS is supported by grants HD 16102 and HD 00719 from the NIH and grant 0551/88 from NATO.

529.23

DIFFERENTIAL EFFECTS OF HYPOXIA VERSUS ANOXIA ON DEVELOPING RAT EEG ACTIVITY. F.E.JENSEN, C.D.APPLEGATE, D.HOLTZMAN, AND J.L.BURCHFIEL. Children's Hospital, Boston, MA 02115

Exposure to hypoxia and/or ischemia during the perinatal period is associated in human infants with a high risk of subsequent neurologic deficit. It is not well known how the immature brain differs from adult in response to hypoxia/ischemia. We compared acute in vivo EEG responses of rats exposed to varing degrees of O_2 deprivation in development to that of the adult. 5 age groups tested were 5-8, 10-12, 15-16, 20-21days old, and adult (50-70). EEGs were recorded before, during, and after exposure to 0,2,3, or 4%O_2. The response to hypoxia was qualitatively and quantitatively different from anoxia. Unlike anoxia, hypoxia did not cause EEG silence in most rats younger than 16 days. Anoxia (0%O_2) caused a steady decline to isoelectric EEG in all ages. Mean latency to flat line decreased with age. A new finding was that hypoxia(3%O_2) induced either tonic clonic seizures or EEG discharges in all 10-11 d.o. rats. More severe hypoxia (2%O_2) was needed to produce EEG discharges in the 5-8 d. o. rats, but no overt seizure behavior was seen. In the 5-8 and 10-12 d.olds, recovery EEGs up to 48hrs after hypoxia showed spike discharges. Hypoxia occasionally caused short discharges in the 15-17 d.olds, but no overt seizures. Hypoxia never induced seizure activity in animals older than 21days. This suggests that hypoxia has a qualitatively different effect from anoxia, and that there is a critical period during development for hypoxic epileptogenesis.

Supported by NIH-NICHD#HD00807 and the Kaplan Foundation.

529.24

THE HYPERGLYCEMIC HORMONE GALANIN BLOCKS THE GLUTAMATE MEDIATED ANOXIC DEPOLARIZATION IN CA3 HIPPOCAMPAL NEURONS.

by Y. BEN ARI & M. LAZDUNSKI*, INSERM U29, Hôpital de Port-Royal 123 Bld de Port-Royal, 75014 PARIS.

Galanin is a hyperglycemic hormone which inhibits insuline secretion from pancreatic B cells by activating K^+ channels normally blocked by intracellular ATP (ATP-K^+ channels). These channels are blocked by sulfonylureas which are widely used as antidiabetic drugs. High affinity binding sites to the potent sulfonylurea glibenclamide (Glib) have been found in the brain notably in the hippocampus (Mourre et al, Brain Res. 1989 (486) 159). We now report that the depolarization induced by anoxia in CA3 neurons is increased by Glib and blocked by galanin.

Brief anoxic episode (1-5 min.) induced in CA3 pyramidal neurons recorded from slices, a large depolarization followed upon reoxygenation by a post anoxic hyperpolarization due to the reactivation by oxygen of the Na^+-K^+ electrogenic pump. Anoxic depolarization is due to an enhanced release of an excitatory amino acid since it was blocked by tetrodotoxin (1 µM) or kynurenate (1 mM). Bath application of Glib (2-5 µM) or galanin (1 µM) during the anoxic episode respectively augmented and blocked the anoxic depolarization. Both agents had littke effect in oxygenated solution suggesting that their action may be mediated by ATP-K^+ channels. We suggest that by blocking the toxic release of glutamate during anoxia, agents such as galanin may be important to prevent neuronal death during and following anoxia.

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NEUROTOXICITY III

530.1

HISTOPATHOLOGICAL EFFECTS OF OMEGA-CONOTOXIN IN RATS. S. Shapira,* T. Kadar* and G. Feuerstein[1]. Dep. Pharmacol., IIBR, Ness-Ziona 70450, ISRAEL, and [1]Dep. Neurology, USUHS, Bethesda MD 20814.

The neurotoxin Omega-conotoxin (w-ctx) is a potent L/N Ca^{+2} channel blocker known to cause distinct toxic effects in conscious rats even at extremely low doses (0.032nmol/kg). The present study was designed to identify sites of neuronal vulnerability to w-ctx. Rats (300-400g) were injected 0.1 or 0.032 nmol/kg w-ctx into the right lateral ventricle, through a previously (7-10d) implanted guide-cannula. Three days later rats were euthanized, perfused with formaldehyde, and the brains removed and embedded in paraffin. Sections (6µm) were stained with H&E, luxol or PHG. Brain lesions were found in the hippocampal formation, the cortex and the hypothalamus. In the hippocampus, damage was ipsilateral and highly selective to CA3 and dentate gyrus (DG), where pyramidal cells underwent shrinkage and degeneration. The extent and severity of the hippocampal injury suggest an irreversible damage. These findings indicate the presence of specific w-ctx sensitive neurons in the hippocampus and DG. Although the histological study was performed after recovery of the rats from the gross motor deficits, the selective neuronal death suggests hippocampal related damage.

530.2

CHLORDECONE EFFECTS ON FOOD INTAKE AND GASTROINTESTINAL MOTILITY IN THE MALE RAT. J. Williams , S. Montanez,* S. Salamanca,* and L. Uphouse. Dept. Biology, Texas Woman's Univ., Denton, TX 76204. Serotonin has been implicated in both the neurological and reproductive effects of the chlorinated pesticide, chlordecone. Serotonin is also involved in regulation of eating behavior and gastric motility; therefore, this study was undertaken to determine the effects of chlordecone on food intake and gastrointestinal (GI) motility. Adult male CDF-344 rats were used for all experiments. Significant decreases in food intake and body weight were seen following treatment with 75 mg chlordecone/kg. However, a greater percentage of ingested food was retained in the stomach. A significant delay in movement of ingested food through the GI tract was observed when animals were injected with chlordecone after eating ad lib. overnight. Weight loss after food removal in animals treated with chlordecone was statistically significant from that of control animals only during the time period when tremor activity was at the lowest level. In conclusion, chlordecone treatment decreases food intake and movement through the GI tract, suggesting that chlordecone may affect the peripheral serotonergic system. Weight loss seen in chlordecone treated animals may be due primarily to decreased food consumption rather than to tremor activity.

(NIH ES03351 grant to LU)

530.3

BINDING OF ^{125}I-TETANUS TOXIN TO RAT BRAIN MEMBRANES. N. M. Bakry*,R.C. Sorensen and L.L. Simpson. Dept. Med.,Div. Environ. Med. and Toxicol., Jefferson Medical College, Philadelphia, PA 19107.

We have compared several methods for labelling tetanus toxin with ^{125}I, including chloramine-T, Bolton-Hunter, Iodobead and Enzymobead techniques. The radioiodinated toxin was subsequently evaluated for neurotoxicity, binding activity and specific activity. The results showed that the Iodobead and Enzymobead methods were unsatisfactory. Incubation with the toxin for sufficient time to give high specific activity resulted in loss of toxicity and binding activity. The chloramine-T method also resulted in substantial loss of toxicity (80-90%), although binding activity was retained. The Bolton-Hunter reagent gave the best result; a preparation with specific activity of 750 Ci/mmole retained 50% or more of its native toxicity and retained the ability to bind to rat brain synaptosomes. ^{125}I-tetanus toxin bound to two sites (Kd= 0.07 nM and B max= 0.4 pmol/mg protein; and Kd= 0.8 nM and B max= 1.5 pmol/mg pr.) These data were obtained by several methods, including saturation isotherm of the specific binding of ^{125}I-tetanus toxin, displacement of specific binding of labelled toxin by unlabelled toxin, and determination of pseudo-first order association rate constant and dissociation rate constant. Analysis of these data confirmed that the binding behavior of ^{125}I-tetanus toxin (Bolton-Hunter reagent) resembles that of unlabelled toxin.

530.4

SELECTIVE NEUROTOXICITY OF CLIOQUINOL ON THE FUNCTION OF THE POSTERIOR COLUMN NUCLEI. K. Arasaki, N. Ohkoshi* and T. Nakanishi*. Dept. of Neurology, Univ. of Tsukuba, Ibaraki-Pref., 305, JAPAN

The pathophysiology to cause dysesthesia in patients with subacute myelo-optico-neuropathy (SMON) is not known. We have studied in rats the physiologic effect of the intraperitoneal administration of clioquinol, a chemical suspected to cause SMON in Japan, for one month. This method produces morphological changes in the rat nervous system comparable to those in SMON (Kotaki, H. et al. J. Pharm. Dyn., 6:773, 1983). After the treatment, rats were anesthetized with alpha-chloralose. The spectrum of the afferent nerve conduction velocities in the sural nerve and the cord dorsum potential evoked by stimulation of this nerve were not affected. However, the amplitude of P wave in the evoked potential recorded on the surface of the gracile nucleus after stimulation of the sural nerve (EP-GN) was markedly diminished in clioquinol treated rats. Furthermore, repetitive stimulation of this nerve revealed that N wave of EP-GN in clioquinol treated rats persisted with shorter stimulation intervals than in control rats. These data showed that dysesthesia in SMON may be produced by a decrease in the presynaptic inhibition in the posterior column nuclei, which amplifies the excitatory synaptic transmission in the nuclei.

530.5

ALUMINUM MALTOL-INDUCED CYTOSKELETAL CHANGES IN FETAL RABBIT MIDBRAIN EXPLANTS IN MATRIX CULTURE. C.D. Hewitt*, M.M. Herman*, C.D. Katsetos*, J. Savory* and M.R. Wills*. (SPON: S.R. VandenBerg) Univ. of Virginia, Charlottesville, VA 22908.

We have evaluated the neurotoxic effects of aluminum (Al) on fetal rabbit midbrain explants using an in-vitro matrix system. Midbrain was explanted at 24 days' gestation and maintained in DMEM-F10 medium with 10% rabbit serum. After 15 days, cultures were treated thrice weekly with aluminum maltol (AM) for 24 or 25 days. Argyophilic neuritic swellings and perikaryal inclusions were produced at 11, 13 and 15 μM AM in 1, 2 and 1 experiments respectively, compared with controls. By immunohistochemistry, neuritic swellings and occasional perikarya exhibited reactivity toward phosphorylated epitopes of neurofilament protein (NF-H/M) but were negative for tau, MAP2 and β-tubulin (hβ4) after similar treatment with 11 and 13 μM AM. Treated cultures, maintained for an additional 10 days without Al retained neuritic pathology. By electron microscopy, focal accumulations of 10 nm filaments were observed in occasional neuronal somata and neurites of treated explants. Our findings are in keeping with the neuronal cytoskeletal changes noted in experimental Al intoxication in-vivo. (Grant # ESO4464-02)

530.6

DFO TREATMENT OF NEUROBLASTMA CELLS EXPOSED TO ALUMINUM IN VITRO. E. Uemura, J. Moneysmith, M. Minachi. Department of Veterinary Anatomy, Iowa State University, Ames, IA 50011.

Effects of deferoxamine (DFO), a trivalent metal-chelating agent, were studied on mouse neurobalstoma cells exposed to aluminum in vitro. Cells were grown as a monolayer on a sterile glass coverslip contained in plastic petridish. Cells were first grown in media that contained 0.05% aluminum tartrate (w/v) for 5 days, then media were changed to the aluminum-free media or the aluminum-free media with 0.02% DFO. Cellular content of aluminum was detected by morrin stain, and cellular processes were measured on photographs taken every 24 hours. The control neuroblastoma cells extended long cellular processes with many branches. However, the majority of the aluminum-treated cells did not extend processes; further, those cells that extended processes were characterized by short processes that rarely branched. Morrin stain of the aluminum-treated cells showed an accumulation of aluminum in the nucleus within a few days, and by day 5 many cells started to die. Cellular content of aluminum was reduced significantly by adding DFO (0.02%) in the media for 5 days, and many cells started to extend the cellular processes. It appears that DFO treatment reverses the effects.

530.7

INHIBITION OF RAT SERUM ESTERASE ACTIVITY. J.P. Chambers* S.L. Hartgraves and M. Murphy. Brain Res. Lab. of Biochem. The University of Texas at San Antonio, San Antonio, TX 78285.

Using the substrates p-nitrophenyl -acetate and -butyrate, we examined the effects of three inhibitors on esterase activity of rat serum. Reaction mixtures contained in a final volume of 100 ul, 0.1 M Phosphate buffer, pH 8.0, 2.5 mM substrate and 10 ul serum. Reactions were carried out at 35 °C for 5 mins and terminated by addition of 600 ul cold 1:1 (v/v) Chloroform:Methanol. Partitioning of liberated phenol was facilatated by addition of 900 ul 2.78 mM NaCl followed by centrifugation of reaction mixtures at 2000 rpm for 5 mins. A 500 ul aliquot of the aqueous phase (upper) was removed and partitioned phenol converted to phenolate anion by treatment with 1.0 ml 0.2 M glycine-ammonium hydroxide buffer, pH 10.5 and monitored spectrophotometrically at 400 nm. Inhibition studies using cresylbenzodioxaphosphorin oxide (CBDP), bis-4-nitrophenylphosphate (BNPP) and tetraisopropyl pyrophosphoramide (iso-OMPA) revealed CBDP affected preferentially hydrolysis of p-nitrophenylbutyrate whereas BNPP inhibited preferentially hydrolysis of p-nitrophenylacetate. The inhibitor iso-OMPA exhibited no inhibitory effects upon hydrolysis of either substrate. Maximum inhibition observed for either CBDP or BNPP was 50 % suggesting the presence of esterase activities preferentially sensitive to two specific esterase inhibitors.

530.8

THE EFFECT OF HYDROGEN SULFIDE ON AMINO ACID NEUROTRANSMITTER RELEASE PATTERN IN RAT BRAINSTEM RETICULAR NUCLEUS. S.B. Kombian*, M.W. Warenycia & R.J. Reiffenstein. Univ. of Alberta, Edmonton, AB, T6G 2H7.

Hydrogen sulfide (H_2S) very toxic. H_2S causes death by paralysis of central respiratory drive. Few neurochemical studies have been done to elucidate its toxicity. Using the push-pull perfusion technique, we studied the effect of NaHS on the release of amino acid neurotransmitters in a brainstem nucleus responsible for maintenance of respiration and cardiovascular homeostasis. Recovered perfusates were separated and quantified by HPLC. NaHS (15mg/kg, IP) altered neither excitatory (GLU & ASP) nor inhibitory (GLY & GABA) neurotransmitter release pattern. It caused, however, a delayed decrease in the release of glutamine, a precursor of glutamate to 61.2±2.9% of control ($p<0.05$, $n=5$). NaHS (2μg/ml, close to endogenous HS^-levels) applied directly into the nucleus for 2 min. produced no change in excitatory or inhibitory transmitter release, but 3μg/ml produced a delayed decrease ($p<0.05$ compared to basal; $n=6$) in release of glycine to 61.6 9.7% of control. Since glycine is the main inhibitory neurotransmitter in brainstem and spinal cord, decreases in its release may represent disinhibition leading to unopposed excitatory events and loss of respiratory drive. (Supported by Occupational Health & Safety Heritage Grant; SBK is an MRC(C) Student).

530.9

HYDROGEN SULFIDE INHIBITS RAT HIPPOCAMPAL CA1 PYRAMIDAL CELLS AND SYNAPTIC POTENTIALS *IN VITRO*: MECHANISM OF TOXICITY? R.J. Baldelli*, R.J. Reiffenstein and W.F. Colmers (SPON: M.W. Warenycia), Department of Pharmacology, University of Alberta, Edmonton, AB, CANADA, T6G 2H7

H_2S has a variety of toxic effects, including psychiatric reactions, amnesia, and paralysis of central respiratory drive. We have studied the mechanism(s) of H_2S action on CNS neurons, using the rat hippocampal slice, *in vitro*.

Hippocampal slices (450 μm) were prepared using standard techniques, and held submerged in oxygenated buffer at 35°C. CA1 pyramidal cells were impaled with 2M K^+ acetate-filled microelectrodes (85-150 MΩ). NaHS (40-160 μM) was dissolved in buffer just prior to application via the bath. [Brain acid-labile S^{2-} is 75 μM at the LD_{50}.]

NaHS (≥ 80 μM) caused a significant ($P<0.05$), concentration-dependent hyperpolarization of CA1 cells, and a further, concentration-related hyperpolarization was often observed just after washout ($P<0.05$ at 160 μM). These changes were seen in low Ca^{2+}, high Mg^{2+} medium, which abolished synaptic transmission, and were often associated with a decrease in membrane input resistance. EPSP's evoked by stimulation of stratum radiatum were significantly ($P<0.05$) attenuated by NaHS at concentrations ≥ 60 μM; 160 μM NaHS reduced the EPSP to 17±3% of control. **All effects of NaHS were rapidly reversible** (5 - 15 min).

Results suggest that inhibition of neuronal activity by H_2S (e.g., retrograde amnesia) may involve suppression of synaptic input and direct hyperpolarization. Similar mechanisms may be operative in the brainstem, and account for the fatal loss of central respiratory drive seen in acute intoxication.

Supported by MRC (Canada) and Alberta Occupational Health and Safety Heritage grants; RJB has an MRC (Canada) Studentship.

530.10

MORPHOLOGICAL AND NEUROCHEMICAL EFFECTS OF CHLORDECONE IN NEURAL TISSUE. M. L. Higgins*, H. Brown*, S. Montanez*, and L. Uphouse. Dept. Biology, Texas Woman's Univ. Denton, TX 76204.

Chlordecone's reproductive effects have often been attributed to its interaction with the intracellular estradiol receptor. To determine if the pesticide mimicked estradiol's induction of rough endoplasmic reticulum (RER) stacking in the ventromedial nucleus (VMN), ovariectomized rats were injected three days with oil, estradiol (10 ug/day), chlordecone (25 mg/kg/day), or estradiol plus chlordecone. The VMN was examined by EM. Estradiol produced an increase in stacking of RER. Chlordecone was ineffective. Estradiol plus chlordecone produced a significant increase in number of cisternae per stack. This suggests that chlordecone's interaction with the estradiol receptor does not antagonize one index of estradiol action in the VMN.

In contrast, serotonergic mechanisms may be involved in chlordecone's neuroreproductive toxicity. The 5-HT1A binding site is reduced in proestrous females treated with chlordecone. Additional evidence confirms this and suggests that the 5-HT1B binding site is increased in chlordecone treated females. The reproductive toxicity may result from a disrupted balance between serotonin receptor subtypes involved in the facilitation and inhibition of reproductive behavior. (NIH ES03351 to LU)

530.11

POSTNATAL DEVELOPMENT OF CATALASE ACTIVITY IN THE OLFACTORY PATHWAY. R. Coopersmith and M. Leon. Dept. of Psychobiology, Univ. of California, Irvine, CA 92717.

Olfactory receptor neurons, located in the nose, are in continuous contact with the environment, exposed to odorants as well as potentially toxic substances. Receptor cells are therefore particularly vulnerable to toxic insult and represent a route of entry to the brain. To determine whether these neurons have active mechanisms to protect themselves and their projection sites, we measured catalase activity in the rat olfactory epithelium and olfactory bulb. Catalase metabolizes hydrogen peroxide to water and oxygen, preventing peroxidative cellular damage.

Catalase activity was assayed in olfactory epithelium, olfactory bulb and occipital cortex from Wistar rats ranging in age from Postnatal Day (PND) 0 (day of birth) to PND 40, by the method of Aebi. We assayed catalase activity in the developing rat as a first step in a study of this enzyme throughout the lifespan. At all ages, activity was highest in olfactory epithelium, lower in olfactory bulb and lowest in occipital cortex. In all three tissues, catalase activity increased during the first week of life, peaking at PND 10, when activity was slightly more than twice that on PND 0. Enzyme activity then declined over the next five days, below PND 0 levels. In bulb and cortex, activity remained low until PND 40, with bulb activity consistently higher than cortex. Catalase activity in olfactory epithelium steadily rose until activity on PND 40 was slightly higher than on PND 0. The small but consistent difference between bulb and cortex levels may be a result of very high activity in the nerve layer, comprising olfactory receptor axons, which represents a relatively small proportion of the entire bulb volume.

530.12

INCREASE IN GLUCOSE-6-PHOSHATE DEHYROGENASE ACTIVITY IN OLFACTORY PATHWAYS IN RESPONSE TO FORMALDEHYDE. S.Miller, R.Coopersmith, and M.Leon. Department of Psychobiology, University of California, Irvine, CA 92717.

Dendritic processes of olfactory receptors lie in the nasal mucosa, outside the protection of the blood-brain barrier making them more easily exposed to environmental toxicants than the rest of the brain. Since it seems that olfactory pathways may be a route for entry of toxicants into the CNS, we examined possible cellular protective mechanisms in this system. Glucose-6-phosphate dehydrogenase (G6PD), the rate-limiting enzyme of the hexose monophosphate shunt, shows unusually high activity in olfactory neurons. The hexose monophosphate shunt produces NADPH required for cytochrome p450 and glutathione detoxification systems. If the hexose monophosphate shunt is involved in detoxification in olfactory pathways, it might respond to an airborne toxicant such as formaldehyde.

Rat pups were exposed to formaldehyde (a 1:1 dilution of a saturated vapor) for 1 hour/day from postnatal day 10 - 20. G6PD activity was then measured in exposed and control rats at postnatal day 20. Exposed rats showed higher G6PD activity in olfactory epithelium and olfactory bulb, while activity in cortex was the same for control and exposed rats. This finding suggests that detoxification systems in olfactory pathways may be modified in response to environmental challenges.

530.13

INCREASED PRESENCE OF GLUTATHIONE IN OLFACTORY EPITHELIUM. R.J. Bridges, M. Anderson* and M. Leon. Depts. of Neurology and Psychobiology, University of California, Irvine CA 92717

The olfactory receptor neurons of the nasal epithelium represent a possible site of entry into the CNS for a wide variety of substances. Dyes, toxins, colloidal gold, HRP, and viruses have all been shown to gain acess to the the brain *via* the olfactory system. For the most part, however, the brain appears to be normally protected from xenobiotics that could enter in this manner, suggesting that efficient detoxification mechanisms are in place in this system. As glutathione has been identified as an essential component of several detoxification schemes, we have examined the differential distribution of glutathione in the olfactory epithelium, olfactory bulb, and other areas of the rat brain.

Brain areas of interest were quickly dissected out and frozen in a dry ice/ethanol bath. The tissues were weighed, homogenized in 5 volumes of 1% sulfosalicylic acid and centrifuged. The glutathione concentrations in the supernants were determined with glutathione reductase and followed the reduction of DTNB (Griffith, 1980).

Substantial levels of glutathione, approximately 2 μmol/g tissue, were detected in all of the regions of the rat brain examined. Moreover, the glutathione content detected in olfactory epithelium was about 50% higher than found in the other brain regions. These results, in combination with increased levels of catalase and glucose-6-phosphate dehydrogenase, identify the olfactory system as particularly enriched in its capacity to detoxify xenobiotics.

530.14

COMPARISON OF THE NEUROPATHOLOGIES OF AMPHOTERICIN B (AB) AND ITS METHYL ESTER DERIVATIVE (AME) IN PERIPHERAL NERVE. B.G. Gold[1], M.T. Ryzlak*[2] and C.P. Schaffner*[2]. [1]Neurotoxicol. Labs., Coll. of Pharmacy and [2]Waksman Inst. of Microbiol., Rutgers Univ., Piscataway, NJ 08854.

Amphotericin B methyl ester (AME) retains antifungal activity but is significantly less nephrotoxic than AB. However, the association of AME with diffuse white matter degeneration has prevented its continued clinical use. The neurotoxicity of these compounds was studied by subepineurial injection (10 ul) of AB or AME (1 mg/ml), or saline, into the rat sciatic nerve using a glass micropipette; glutaraldehyde (5%) fixed tissues were examined between 1-14 days. In AME-injected nerves, reactive axonal swellings with distal axonal atrophy (suggesting axostasis) and myelin bubbles were present at 1 day; by day 2, axonal degeneration (1-2%) was found. Extensive active segmental demyelination with myelin phagocytosis by macrophages was observed at 2 and 7 days; remyelination and axonal sprouts were evident at 7 days. AB produced similar changes, albeit with more pronounced degeneration, less regenerating sprouts and remyelinating axons. At 14 days, most fibers demonstrated somatofugal (proximal) axonal atrophy. In AME-treated nerves, this axotomy-like response was more prominent than axonal loss and may arise via an alteration in trophic mechanisms due to impaired axonal transport and/or demyelination-induced conduction block. (Supported by NS26265).

531.1

PROCYCLIDINE PROTECTS AGAINST SOMAN NEUROTOXICITY, EVEN WHEN ADMINISTERED AFTER ONSET OF CONVULSIONS. M.T. Price, G.R. Stewart and J.W. Olney, Washington University School of Medicine, St. Louis, MO.

Soman, an organophosphate cholinesterase inhibitor, induces a devastating neurotoxic syndrome featuring persistent seizure activity and wide-spread seizure-related brain damage. A major problem in studying this syndrome is posed by the marked individual variation in sensitivity of experimental animals. Some adult rats develop status epilepticus only minutes after receiving a dose of soman in the range of 90 - 125 µg/kg and such animals typically sustain severe brain damage and die, yet others can tolerate much higher doses without experiencing seizures, brain damage or death. Administering LiCl 24 hours prior to soman is advantageous as it moderately, but consistently, increases the percentage of animals susceptible to soman neurotoxicity.

In the present study, we evaluated the ability of procyclidine to protect against Li/soman neurotoxicity. Procyclidine is an anti-Parkinsonian drug with anti-cholinergic properties which also antagonizes the N-methyl-D-aspartate (NMDA) receptor (a subclass of glutamate receptor hypothetically implicated in seizure-related brain damage). The individual variability problem was obviated by the following research design: all animals were pretreated with LiCl (3 meq/kg sc) and 24 hrs later given soman (125 µg/kg sc) and observed for symptoms; animals that began convulsing were treated immediately either with saline or a single dose of procyclidine (75 mg/kg ip). All animals were killed 4 hours after soman treatment and their brains examined histologically. Rats that did not seize (n = 28) did not have any brain pathology. All rats that seized and received saline (n = 8) had severe disseminated brain damage. Rats that seized and received procyclidine (n = 12), stopped seizing within 5 to 15 minutes; all of these rats escaped brain damage. When atropine (up to 100 mg/kg ip) was substituted for procyclidine in the above protocol, it conferred no protection against soman neurotoxicity. While it is unclear whether the protective action of procyclidine can best be explained in terms of an anticholinergic or NMDA antagonist mechanism (or both), it is significant that procyclidine prevented soman neurotoxicity even when given after neurotoxic symptoms had become manifest.

Supported by RSA MH 38894 (JWO), ES 07066 and DAMD 17-86-C-6010.

531.2

LONG-TERM POTENTIATION IS REDUCED IN RATS PRETREATED WITH LOW DOSES OF SOMAN. **D. L. Armstrong, T. Osaka, S. A. Miller*, S. Z. Kerenyi* and M. R. Murphy. Division of Life Sciences, The University of Texas at San Antonio, San Antonio, TX 78285 and USAFSAM, Brooks AFB.**

Rats exposed to sublethal doses of soman display a number of symptoms that include weight loss, dehydration, hypothermia and hyperreactivity. In these experiments the magnitude and duration of long-term potentiation (LTP) within the dentate gyrus following tetanic stimulation of the perforant path was compared between soman treated and weight loss control rats.

The amplitude of population EPSPs evoked by a test pulse was measured in urethane anesthetized rats before and after a series of tetanic stimulation trains were applied to the perforant path. Control animals (n=5) displayed 100% increases over baseline EPSP amplitudes of 2.2 ± 0.4 mV (x ± S.D.) which were maintained for the duration of the three and a half hr. recording sessions. Two to three weeks after soman exposure, experimental animals (n=5) displayed much greater variability in baseline EPSP amplitudes and, either no potentiation of response followed tetanic stimulation, or a small amount of potentiation (>50%) occurred which was not maintained for the full three hours. Loss of potentiation was observed in animals who had recovered from other symptoms such as hyperreactivity. These results indicate that disruption of physiological mechanisms associated with learning and memory functions occurs in animals exposed to low doses of soman.

Supported by Air Force Contract No. F33615-87-D-0609.

531.3

A CENTRALLY ACTING NOVEL TERTIARY PYRIDOSTIGMINE DERIVATIVE 3-(N,N-DIMETHYLCARBAMYLOXY)-1-METHYL-Δ^3-TETRAHYDROPYRIDINE (THP) IN COMBINATION WITH PYRIDOSTIGMINE PROTECTS AGAINST SOMAN TOXICITY. R. Ray, O.E. Clark*, K.W. Ford*, K.R. Knight*, L.W. Harris* and C.A. Broomfield*. US Army Medical Research Institute of Chemical Defense, APG, MD 21010-5425.

Reversible inhibition of acetylcholinesterase (AChE) in both central and peripheral compartments may be required for protection against soman intoxication. We reported earlier (FASEB J. 3(3):A893, 1989) that THP, im, penetrates the blood-brain barrier and inhibits AChE in both blood and brain, whereas pyridostigmine (Pyr), im, inhibits AChE in the blood only. Here we report that im pretreatment of guinea pigs with THP (262 ug/kg) plus Pyr (131 ug/kg), 30 min prior to sc soman challenge, with no antimuscarinic or oxime treatment protects 60% of the animals against 2xLD_{50} of soman. Inhibition of AChE in the blood (15%) and in the brain (30%) by THP (262 ug/kg) alone, or in the blood (70%) only by Pyr (131 ug/kg) alone provide no protection. The protective pretreatment regimen does not prevent convulsions, but significantly shortens the recovery time in surviving animals (median recovery time 1.6 hr, compared to 24 hr in control and other groups). A combination of THP and Pyr thus appears to provide a means to evaluate the relative importance of selective peripheral plus central vs peripheral AChE protection against soman.

531.4

SOMAN INDUCED BRAIN DAMAGE: PROTECTION PROVIDED BY DIAZEPAM. S.K. Campbell*[1], R.C. Switzer III[1], M.R. Murphy[2], S.Z. Kerenyi*[3], S.A. Miller*[3], and S.K. Hartgraves[3] (SPON: N. Greenberg). [1]R.H. Cole Neuroscience Lab., Dept. of Pathology, Univ. of Tennessee Med. Ctr., Knoxville, TN 37920; [2]Systems Res. Lab. & [3]Radiat. Sci. Div., USAF Sch. Aerosp. Med., Brooks AFB, TX 78235.

Soman is an irreversible, anticholinesterase (AChE), organophosphate neurotoxin. Using the degeneration-sensitive cupric-silver histological method of deOlmos, damage to the central nervous system (CNS) from both acute and chronic low doses of soman has recently been shown in chemically unprotected rats (Soc. Neurosci. Abstr. 14(1):774). In this study we sought to detect CNS damage using the cupric-silver method in rhesus monkeys that were also evaluated for motor performance deficits (Glick et al, these proceedings). All monkeys were pretreated with pyridostigmine via minipump and received an acute dose (3xLD50, im) of soman while provided with protective therapy of atropine and 2-PAM (an AChE reactivator). One half of the animals also received diazepam as an anticonvulsant. The animals were sacrificed after 1 or 14 weeks. In the first two monkeys examined, extensive degeneration was found in the non-diazepam treated monkey, after one week, that was comparable to the damage we have observed in rats. The areas affected include: amygdala, piriform cortex, hippocampus, select thalamic nuclei and subpopulations of cortical neurons. Brain stem, hypothalamus and upper spinal cord were spared. In the diazepam treated monkey no damage was detectable after 14 weeks of survival.

Performed under USAF Contract F33615-87-C-0625 with funding from the USAMRIDC.

531.5

EFFECTS OF DIAZEPAM ON SOMAN-INDUCED LETHALITY, CONVULSIONS, AND PERFORMANCE DEFICIT. D. W. Blick*, M. R. Murphy*, J. W. Fanton, S. Z. Kerenyi, S. A. Miller and S. L. Hartgraves. *Systems Research Laboratories, and USAF School of Aerospace Med., Brooks AFB, TX 78235

In 12 rhesus monkeys well trained on the Primate Equilibrium Platform (PEP) task, we tested performance recovery after exposure to 3 times the estimated LD_{50} of soman on days .5, 1, 2, 3, and 6 following exposure, and weekly thereafter for 13 weeks. All subjects received pyridostigmine before, and atropine and 2-PAM after soman exposure; half of them also received diazepam after soman.

Diazepam was found to decrease significantly the duration of soman-induced convulsions. Survival was more likely in diazepam-treated subjects (89%) than in non-diazepam treated subjects (50%), but this effect was not statistically significant due to the small n involved ($p<.30$, using χ^2).

For subjects that survived for the 12 hr and 24 hr post-soman performance tests, diazepam conferred a significant relative protection against the soman-induced performance deficit. For animals that survived for the full 13 weeks of post-soman testing, diazepam treatment had no overall effect on performance, but tended to shift the performance deficit to a later period in time. Recovery of performance continued throughout the 13 weeks.

Performed under USAF Contract F33615-87-D-0627/002 with funding from the USAMRICD.

531.6

CLOSED SKULL COBALT 60 STEREOTACTIC RADIOSURGICAL LESIONS OF THE PRIMATE BRAINSTEM. E.M. Altschuler*, L.D. Lunsford, J.C. Flickinger*, A.J. Martinez, R. Sclabassi. University of Pittsburgh School of Medicine, Pittsburgh, PA 15213

To define the radiation tolerance of the brainstem in an animal model, we have correlated serial in vivo neurodiagnostic imaging and electrophysiological studies after closed skull 8 mm radiosurgical lesions of the ventral pons. Six anesthetized baboons (Papio cynocephalus/anubis) had 8 mm maximal diameter single dose radiosurgical lesions produced by the 201 source Cobalt 60 gamma knife. Serial imaging techniques (magnetic resonance imaging and computed tomography) define the rate and extent of lesion development, verified by postmortem examination. Both high dose (150 Gy) and low dose (20 Gy) pontine lesions rapidly alter wave 5 of the brainstem evoked response with preservation of earlier waves. Somatosensory evoked responses are affected if the lesion encroaches on the lemniscal pathway. Precise 4 or 8 mm stereotactic lesions can be performed in the animal brainstem without a surgical incision.

531.7

INTRAOPERATIVE RADIATION THERAPY PRODUCES MASSIVE HEMORRHAGE IN CANINE SPINAL CORDS.
A.M.DeLuca*, W.J.Anderson*(1), T.J.Kinsella, and W.F.Sindelar. Radiation Oncology and Surgery Branches, National Cancer Institute, N.I.H., Bethesda, MD 20892 and (1) Indiana University School of Medicine, Terre Haute Center for Medical Education, 135 Holmstedt Hall, Terre Haute, IN 47809.

This study was part of an ongoing investigation of intraoperative radiation therapy (IORT) using the canine model and is intended to provide guidelines for IORT in various protocols utilized at the NCI and elsewhere. The retroperitoneal structures of normal canines were treated with external radiation therapy (XRT) of 15 MeV photons, total dose of 3,000 rad given in ten fractionated doses of 300 rad each. The ten treatments were given over a period of 4 weeks. The XRT was administered with bilateral 6.5 x 8.5 cm. fields to the midline. A total of 36 female beagles 12-18 months of age were utilized. The dogs displayed no overt effect such as radiation sickness due to the experimental procedures. At 12 months, the majority of animals were displaying a loss of tail reflex, rear end paralysis and incontinence. A total of 15 animals whose spinal cords were removed and prepared for tissue analysis demonstrated severe hemorrhage between the L5-S2 segments. Every animal showed severe demyelination (in some cases leukomalacia) which indicates the XRT doses are too large for spinal cord exposure under this protocol.

531.8

NAD(P)H:QUINONE REDUCTASE AND GLUTATHIONE S-TRANSFERASE EXHIBIT REGIONAL DIFFERENCES IN LEVELS AND INDUCTION IN RAT BRAIN. L. McIntosh*, P. Schwartz* and M.J. DeLong*. (SPON: V. Hoellding) Dept. of Environmental Health Sciences, Johns Hopkins School of Hygiene, Baltimore, MD 21205

NAD(P)H:quinone reductase(QR) is protective against glutamate toxicity in N18RE105 neuronal cells. High levels of QR and glutathione S-transferase (GST), the conjugating enzyme of glutathione, are also protective against toxins, mutagens and carcinogens in peripheral tissue. We have examined regional differences in basal and induced levels of these two enzymes in rat brain as possible determinants in neuronal tissue of selective cell vulnerability to toxins and degeneration.

We report significant differences in cytosolic specific enzyme activity levels among the six brain regions assayed with dichlorophenolindophenol for QR and chlorodinitrobenzene for GST. QR levels vary from 270 nmol/min/mg protein in the cerebellum (similar to liver values) to only 40-50% of this level in the cortex, striatum and substantia nigra (regions at risk in Alzheimer's, Huntington's and Parkinson's diseases). The GST levels are highest in the hippocampus with 285 umol/min/mg (1/4 liver levels) followed by substantia nigra, striatum, brainstem, and cortex with 80% of maximal value and cerebellum only 50%. In animals fed enzyme inducing diets of 0.75% t-butylhydroxyanisole or t-butylhydroquinone, or 0.05% Sudan I only QR was induced in the hippocampus, brainstem and cerebellum. No induction of GST was observed for any brain region dissimilar to the usual coinduction of these enzymes in peripheral tissue by these inducers.

We speculate the brain regional differences in levels of the protective enzymes QR and GST or their ability to be induced during toxic challenge may be a determinant of selective neuronal degeneration.

531.9

NEUROPEPTIDE CHANGES IN A PRIMATE MODEL (*Cebus Apella*) FOR TARDIVE DYSKINESIA. P. Johansson*, L. Terenius*, L. Gunne* (SPON: D. Ottoson). Psychiatric Research Center, University of Uppsala, S-750 17 Uppsala, Sweden

Tardive dyskinesia (TD), a potentially irreversible syndrome of involuntary movements following long-term neuroleptic administration, has been connected with reduced GABA functions in the basal ganglia. Decreased activity of glutamate decarboxylase (GAD) was seen in the substantia nigra, the subthalamic nucleus and the medial segment of globus pallidus in a primate model (*Cebus apella*) of TD (Gunne et al. 1984, Nature 309,347). In view of the possibility that peptides are involved in neuroleptic-induced dyskinesias substance P (SP) and dynorphin 1-17 (DYN A) levels were measured in the basal ganglia of the *Cebus apella* model for TD. Regional GAD activities were monitored in these brains as before.

A significant TD-related decrease in GAD activity was found in the subthalamic nucleus, together with nonsignificant reductions in the medial segment of globus pallidus and substantia nigra. Cebus monkeys with an intact GABA system (neuroleptic-treated controls without dyskinesia) showed increased levels of SP in the striatum. On the other hand, the dyskinetic monkeys, with a defective GABA system, did not show a similar rise in striatal SP. DYN A showed no dyskinesia-related changes.

In conclusion, the difference in GAD activity between animals developing dyskinetic symptoms vs. those who did not, was reflected on the neuropeptide side by changes in SP levels.

531.10

SEROTONERGIC CHANGES FOLLOWING TREATMENT OF PROESTROUS RATS WITH P,P'-DDT. **(SPON. J. Hines) L. Uphouse, D. Croissant*, R.Richards Hill*, K. Eckols*, and G. Stewart*. Dept. Biology and Chemistry, Texas Woman's Univ. Denton, TX 76204.**

The neurological effects of p,p'-DDT are thought to result from a delayed closing of the sodium channel following spike generation. In studies, p,p'-DDT was shown to inhibit female rat sexual behavior possibly as a result of enhanced transmitter release. The effects of p,p'-DDT on the serotonergic system was examined in cortex, hippocampus and hypothalamus. Proestrous female rats were treated with oil, 25 mg/kg or 75 mg/kg p,p'-DDT and were sacrificed that evening. There was a dose dependent decrease in 5-HT levels in cortex and in hippocampus but elevations in 5-HIAA were present only in the hypothalamus and only at the higher dose of p,p'-DDT. When hypothalamic slices were perfused in vitro with p,p'-DDT, the compound produced an increase in serotonin release. ^{3}H-8-OH-DPAT binding to the 5-HT1A site, examined in hippocampus and cortex, was complex. The dose of 25 mg/kg p,p'-DDT produced an increase in the Bmax for binding to cortical and hippocampal membranes. Females given 75 mg/kg p,p'-DDT showed a binding profile that was impossible to analyze by linear or nonlinear regression. The results of the in vivo and in vitro studies indicate a disturbance of the serotonergic system of female rats after treatment with p,p'-DDT. (NIH ES03351 grant to LU)

531.11

NEUROTOXICITY OF A SEROTONIN (5-HT)-DERIVED TRYPTAMINE-4,5-QUINONE (4,5-DKT). J.C.Chen,J.Fishman,P.Schnepper,A.To, R.Fine and L.Volicer. Depts. Pharmacol. and Biochem. Boston Univ. Sch. Med., Boston, MA 02118; Dept. Pharmacol. U.Mass, Worcester, MA 01655; ENRMem. Vet. Hospital, GRECC, Bedford, MA 01730.

Previous studies have shown that 4,5-DKT had a stimulatory effect on basal 5-HT efflux in central 5-HT neurons and caused selective terminal degeneration in the medial limbic system. Recent studies demonstrated lateral ventricle 4,5-DKT administration decreased 5-HT and its metabolite 5-hydroxyindoleacetic acid (5-HIAA), quanosine, xanthine and tryptamine in various areas of rat brain sacrificed 4 days later. The decrease in 5-HT and 5-HIAA was not due to the destruction of 5-HT terminals since the uptake of 5-HT into brain synaptosomes remained intact. However, a compensatory increase in 5-HT turnover and altered tryptophan hydroxylase activity was observed. In crude membrane preparation incubated with [^{3}H]4,5-DKT, two bands with kD corresponding to alpha and beta subunits of G-protein were labelled in the SDS-PAGE gel.4,5-DKT strongly inhibited pertussis toxin-catalyzed AD[^{32}P] ribosylation while 5,6-DHT and 5,7-DHT had only minor effects. This effect of 4,5-DKT was inhibited by glutathione. In primary neuronal culture from 9 day chick embryos, cultured for 4 days 4,5-DKT showed dose-dependent toxicity. Glutathione, surprisingly potentiated the neurotoxicity of 4,5-DKT. (Supported by USPHS AG06419 and Veterans Administration).

531.12

PHARMACOLOGICAL VALIDATION OF A NEUROBEHAVIORAL TEST BATTERY IN SPRAGUE DAWLEY (SD) RATS. S.R. Morton*, G.C. Haggerty, S.C. Gad*. G.D. Searle & Co., Skokie, IL. 60077.

A neurobehavioral test battery, consisting of a functional observational battery and an automated test of motor activity, is being developed for incorporation into rodent toxicology studies. The present work describes the first phase of validation testing which was conducted using a variety of pharmacologic agents including amphetamine, chlorpromazine and ethanol. In all studies, SD rats received a single i.p. dose of the test article and were evaluated at the time of peak effects. Amphetamine treated rats showed increased touch response, and open field(OF) and home-cage(HC) activity, as well as tiptoe walking, piloerection and stereotypic behavior. In contrast to this, decreases in OF and HC activity and arousal, increased hindlimb footsplay, and impaired mobility and rotarod performance were seen with chlorpromazine, while ethanol exposure produced decreased body temperature, impaired rotarod performance and ataxia. In summary, these studies demonstrate that the neurobehavioral test battery developed in this laboratory is capable of defining characteristic behavioral profiles of a number of diverse pharmacologic agents.

531.13

NEUROBEHAVIORAL EFFECTS OF REPETITIVE MARIHUANA SMOKE EXPOSURE IN RATS. M.J. Kallman, A.B. Jones*, S.F. Ali, A.C. Scallet, M.G. Paule and W.S. Slikker, Jr.* Depts. of Psychol. & Pharmacol., U. of Miss., University, Ms. 38677 and Div. Reprod. & Dev. Toxicol., NCTR, Jefferson, AR. 72079.

This investigation explored the consequences of 30 days of repetitive marihuana (NIDA cigarettes, 2.85% Δ^9-THC) smoke exposure in a Walton Horizontal Smoke Exposure System. Animals were exposed daily to one of the following treatments: placement in the smoking apparatus but no smoke exposure (NSC), exposure to three placebo cigarettes (3P), exposure to two placebo cigarettes and 1 marihuana cigarette (2P1M) or exposure to one placebo and two marihuana cigarettes (1P2M). Body weights and body temperatures were monitored weekly. Behavioral assessments were conducted 60 days after exposure terminated and included rotarod performance, free-field observation, muscular coordination, and locomotor activity. Amino acid levels were determined in the caudate, cerebellum, and brain stem and GABA and benzodiazepine receptor binding we determined for the hippocampus and frontal cortex. Body weights were significantly reduced by day 40 for the 1P2M treatment group and body weights remained lower throughout the measurement period. Muscular coordination and rotorod performance were also reduced for rats exposed to marihuana. Measures of brain function were not altered by exposure to marihuana smoke. Supported by DA03652.

531.14

TRIMETHYLTIN (TMT) EFFECTS ON HIPPOCAMPAL PHENCYCLIDINE RECEPTORS : DOSE-DEPENDENT DECREASES IN CA AND INCREASES IN DENTATE. R.B. Messing and L. M. Aanonsen[a]. Depts. of Pharmacol., and Cell Biol. and Neuroanat.[a], U. of Minn. Med. Schl., Minneapolis., MN 55455.

TMT selectively damages hippocampus and related structures, but different sequelae result after 6.0 mg/kg or 7.5 mg/kg doses to male LE rats. At the lower dose, TMT produces cognitive and performance impairments reminiscent of aged organisms, and hippocampal corticosterone receptors are decreased, while forebrain ß-receptors are increased. The higher dose causes a larger lesion and may result in irritability and convulsions, but rats have more normal values in biochemical assays. These rats have learning and performance deficits similar to those of animals with large hippocampal lesions[1,2].

Hippocampal NMDA receptors have been implicated in the etiology of brain damage after various insults, and as an important mediator of learning and memory processes. Thus, a quantitative autoradiographic binding analysis of [^{3}H]TCP, the phencyclidine analog and non-competitive antagonist of NMDA, was performed in rats given TMT (6.0 or 7.5 mg/kg p.o.) or water 3 weeks before being killed. At the lower dose, TCP binding was reduced in dentate hilus-CA3, but was unaffected to enhanced in CA1 and dentate. In contrast, TCP binding was reduced throughout the entire extent of Ammon's Horn in high dose rats, but binding was markedly enhanced in dentate gyrus. These effects thus indicate not only differential severity of cell loss, but also varying regulatory or adaptational changes after different doses of TMT, and may be related to the qualtitatively different syndromes which result from each dose. More generally, different levels of CNS damage to the same area may result in widely divergent consequences, and TMT may be a valuable tool for investigating dementing processes at different severities. Supported by ONR N00014-86-K-0407 (RBM) and USPHS F32 DA053309 (LMA). 1) Messing, et al., Neurotoxicol. 9(1988):491. 2) Gerbec, et al., Brain Res. 460(1988): 346.

SYNAPTOGENESIS: NEUROMUSCULAR JUNCTION

532.1

SYNAPTIC PLASTICITY IN SKELETAL MUSCLE: CHANGES IN THE GOLGI APPARATUS DISTRIBUTION DURING DEVELOPMENT AND AFTER DENERVATION. P.J. Jasmin[1], J. Cartaud*[1], M. Bornens*[2] and J.-P. Changeux[3]. Institut Jacques Monod, CNRS, Paris[1], Centre Génetique Moléculaire, Gif-sur-Yvette[2], et Institut Pasteur, Paris[3], France.

In the course of studies about the cellular and molecular mechanisms of synapse genesis, we carried out an immunofluorescence study of the distribution of the Golgi Apparatus (GA) in chick skeletal muscle in primary culture and after endplate formation in 15 day-old chicks. Using a monoclonal antibody against GA, we confirmed the known distribution of the GA in myogenic cells: a juxtanuclear polarized organization in myoblasts and a perinuclear non-polarized one in myotubes. The innervated anterior latissimus dorsi (ALD) muscle displayed a focalized distribution of GA which was restricted to areas located underneatn the motor endplates identified via alpha-bungarotoxin fluorescent labeling of the acetylcholine receptor (AChR). Five days following denervation of the ALD muscle, a striking reorganization of GA took place. The GA then showed a perinuclear distribution in close association with every nucleus of the muscle fibres as observed in myotubes. Also, changes in the organization of microtubules (MT) were noted which coincided with those of GA. The focal distributions of GA and MT in intact muscle fibres and their remodeling upon denervation is interpreted in terms of local synthesis, processing and routing of AChR to the endplate and of the regulation of these processes by motor innervation.

532.2

REGULATION OF INTERSTITIAL CELLS AND MATRIX MOLECULES IN DENERVATED MUSCLE. E.A. Connor, Univ. of Mass. Amherst, MA.

Denervation of skeletal muscle results in a selective accumulation of interstitial cells in junctional regions. These interstitial cells, fibroblasts, may play a role in regeneration of neuromuscular junctions; fibroblasts make extracellular matrix which is directly involved in synapse formation. In fact the accumulation of interstitial cells in denervated frog muscle is accompanied by remodelling of the matrix environment. Denervated junctional regions displayed enhanced interstitial staining with antibodies against fibronectin, N-CAM and tenascin, when compared to the pattern of stain in innervated muscles.

To determine the factors that regulate the appearance of the institial cell accumulation and the remodelling of the matrix after denervation, I have examined whether blockade of muscle activity is sufficient to initiate these denervation responses. Activity was blocked postsynaptically by α-bungarotoxin. Interstitial cell density was determined for junctional and extrajunctional regions of muscles. In controls, the junctional cell density of denervated muscles was 3X that of innervated muscles and remodelling of the junctional matrix was observed. In innervated muscles treated with toxin there was neither a junctional accumulation of interstitial cells nor a denervation pattern of staining for fibronectin or tenascin. Therefore, the junctional accumulation of interstitial cells and matrix remodelling are not triggered simply by loss of muscle activity.

532.3

INTRACELLULAR CALCIUM LEVELS IMMEDIATELY AFTER NERVE-MUSCLE CONTACT.
S.H. Young, S.H. Hulsizer*, A.D. Grinnell.
Jerry Lewis Neuromuscular Research Center,
UCLA School of Medicine, Los Angeles, CA 90024.

A report that contact of positively charged beads with cultured Xenopus muscle cells will produce transient changes in muscle cell calcium within seconds (Zhu and Peng, Dev. Biol. 126:63) prompted us to monitor intracellular calcium levels following nerve-muscle contact in these same Xenopus cultures.

Nerve cells were manipulated into contact with the muscle cells at various points. Chow and Poo (J. Neurosci. 5:1076) have shown that transmitter release can begin within minutes after such contact.

The cells were loaded with the calcium indicator FURA-2 before they were pushed into contact. Cell fluorescence was monitored with a SIT camera connected to a video frame grabber. The first sample was taken 5 secs after contact. In contrast to experiments with beads, no large changes in calcium levels were observed after nerve-muscle contact.
Supported by Grants from MDA, NSF, and NIH.

532.4

FORMATION AND SURVIVAL OF A POSTSYNAPTIC SPECIALIZATION IN CULTURES OF EMBRYONIC NERVE AND MUSCLE CELLS. P.L. Samuels* and M.W. Cohen (SPON: G. Mandl). Dept. of Physiol., McGill University, Montreal, Quebec H3G 1Y6.

Sites of high acetylcholine receptor density at neurite-muscle contacts were stained with fluorescent α-bungarotoxin and followed daily for up to 15 d in cultures of embryonic Xenopus spinal cord neurons and myotomal muscle cells. The great majority (>90%) of these neurite associated receptor patches (NARPs) formed within 1 d of neurite-muscle contact. New ones continued to form as long as the neurites continued to grow and establish new contacts. Based on their length, intensity and area x intensity NARPs reached maturity within 2 d of neurite-muscle contact and changed little thereafter. Almost all (>95%) of them survived as long as the contact survived.

Only rarely did the length or area x intensity of all mature NARPs on the same muscle cell change in the same direction at the same time. Changes in intensity in the same direction for NARPs on the same muscle cell were more common. It is concluded that to a large extent the regulation of NARPs, even on the same muscle cell, is a local phenomenon. Global regulation, for example by a change in the rate of synthesis or degradation of acetylcholine receptors throughout a single muscle cell, apparently plays a less important role. (Supported by MRC of Canada.)

532.5

INTERACTIONS OF NEURONS WITH NORMAL AND VARIANT C2 MUSCLE CELLS IN CULTURE. M. T. Lupa,* H. Gordon, and Z. W. Hall. UCSF, Department of Physiology, San Francisco, CA 94143

Myotubes of the C2 mouse muscle cell line were cultured with mouse spinal cord (SC) or chick ciliary ganglion (CG) neurons. We used immunofluoresence with alpha-bungarotoxin and antibodies to pre- and postsynaptic components to characterize the contacts between neurites and C2 myotubes. These contacts were then compared with those formed on two genetic variants of the C2 cell line: R^-, which synthesizes reduced amounts of acetylcholine receptor (AChR); and S27, which makes reduced amounts of sulfated proteoglycans.

Neurites of both SC and CG cells showed staining either with antibodies to neurofilament protein (NF) or to synaptic vesicle protein (SV), but usually not to both. In wild-type C2 co-cultures, myotubes were preferentially associated with SV-containing neurites as opposed to NF-containing neurites. This preference was also found in co-cultures with the R^- variant, but was not found with S27, the proteoglycan-defective variant, which either showed no preference or a slight preference for NF-containing neurites.

Nerve-muscle contacts in wild-type co-cultures were characterized by AChR clusters that were associated with clusters of 43 kDa protein, as well as clusters of the extracellular matrix components, laminin and JS-1. Nerve-muscle contacts in R^- co-cultures showed none of these specializations while those in S27 co-cultures did.

Muscle cell variants thus promise to be useful tools for dissecting the molecular basis of synaptogenesis.

This work was supported by grants from the NIH and the MDA.

532.6

AGRIN DOES NOT INDUCE ACHR CLUSTERS IN A VARIANT MUSCLE CELL. H. Gordon & Z. W. Hall. Dept. of Physiology S-762, Univ. Calif. School of Medicine, San Francisco, CA 94143.

Cultured myotubes of the C2 mouse muscle cell line spontaneously cluster acetylcholine receptors (AChRs) on their surface. We have isolated a variant subline, S27, that expresses AChR on its surface but does not form spontaneous clusters of AChR. In co-culture of S27 with cells from mouse spinal cord or chick ciliary ganglion, AChR clusters do form near sites of contact with neurites.

Agrin isolated from Torpedo electric organ has been shown to induce AChR clusters in chick and amphibian muscle cultures (Nitkin et al. (1987), JCB 105: 2471). We show here that agrin also induces AChR in primary cultures of mouse muscle and in cultures of the C2 mouse muscle cell line. Agrin does not induce clusters in the variant S27.

The failure of agrin to induce clusters in S27 suggests that the basis of neural clustering of AChR requires neural factors other than, or in addition to, agrin.

We thank B. Wallace and U. J. McMahan for their generous gift of agrin and for their advice.

532.7

CONCANAVALIN A INHIBITS AGRIN-INDUCED FORMATION OF AChR, AChE, AND HSP AGGREGATES. K.W.K. Tsim* (spon: U.J. McMahan). Department of Neurobiology, Stanford University School of Medicine, Stanford, California 94305.

Agrin, a protein isolated from Torpedo electric organ, induces the formation of aggregates of acetylcholine receptors (AChRs), acetylcholinesterase (AChE), and heparan sulfate proteoglycan (HSP) on chick myotubes in culture. In this study, concanavalin A (Con A) was used to examine the role of lateral migration in the formation of AChR, AChE and HSP aggregates. Con A has been shown to immobilize AChRs and so prevent their redistribution. Con A also binds to AChE and so might prevent its lateral migration as well. Pre-incubation of chick myotubes with Con A inhibited agrin-induced AChR aggregation, as expected. Incubation with Con A also reduced the rate of dispersal of AChR aggregates after removal of agrin. Adding Con A together with agrin inhibited not only agrin-induced AChR aggregation, but also agrin-induced accumulation of AChE and HSP. Succinyl Con A, which has little effect on the mobility of AChRs, also inhibited the formation of AChR, AChE and HSP patches. Con A inhibition of agrin-induced aggregation of AChE and HSP, but not of AChRs, was reversed by increasing the amount of agrin added to the culture. These results suggest (1) that AChE does not accumulate in agrin-induced specializations by lateral migration and (2) that Con A not only prevents aggregation of AChRs by immobilizing them, but also prevents aggregation of AChRs, AChE, and HSP by acting as a competitive inhibitor of agrin.

532.8

TWO PATHWAYS FOR AGRIN-INDUCED ACCUMULATION OF AChRS, AChE, HSP AND OTHER POSTSYNAPTIC COMPONENTS. B.G. Wallace, Department of Neurobiology, Stanford University School of Medicine, Stanford, CA 94305.

Agrin, a protein extracted from the electric organ of Torpedo californica, causes the formation of specializations on chick myotubes in culture that resemble the postsynaptic apparatus at the vertebrate skeletal neuromuscular junction. The specializations contain cytoplasmic (43 kD AChR-associated protein), membrane (AChRs and globular forms of AChE and BuChE) and extracellular matrix-associated (heparan sulfate proteoglycan [HSP] and A_{12} asymmetric AChE) proteins. AChRs accumulate in agrin-induced specializations by lateral migration of receptors already in the myotube membrane at the time agrin is added. To determine whether other postsynaptic components accumulate by a similar mechanism, we examined the effects of inhibition of protein synthesis and secretion. We found that agrin-induced accumulation of AChRs and the 43 kD AChR-associated protein is not blocked by inhibitors of protein synthesis or secretion, indicating that the 43 kD protein, like AChRs, accumulates by redistribution of pre-existing molecules. Agrin-induced accumulation of HSP, AChE, and BuChE was blocked by inhibitors of protein synthesis and secretion, indicating that accumulation of these components does not occur by redistribution but requires insertion and/or release of newly synthesized proteins. Thus, different components of the postsynaptic apparatus accumulate in agrin-induced specializations by different pathways.

532.9

CULTURED CHICK MYOTUBES EXPRESS AGRIN-RELATED MOLECULES. Erich Lieth and Justin R. Fallon. Worcester Foundation for Experimental Biology, Shrewsbury, MA 01545.

Agrin, an acetylcholine receptor aggregating molecule isolated from Torpedo, is concentrated at the neuromuscular junction. We have previously shown that molecules antigenically related to agrin are localized at acetylcholine receptor clusters (AchRC) on aneural muscles in vivo. Here we report that muscle-derived agrin can be detected on the surfaces of cultured myogenic cells as early as 24 hours after plating and continues to accumulate for at least 2 weeks, as judged by immunofluorescence microscopy and radioimmune assay. Muscle-derived agrin is also expressed in cultures grown in serum-free media. A portion of the muscle-derived agrin colocalizes with spontaneous AchRCs. Agrin-related molecules derived from muscle are also present at motoneuron-induced AchRCs in nerve/muscle co-cultures.

Taken together, these results indicate that muscle-derived agrin, (perhaps in concert with agrin from nerve, Magill-Solc and McMahan, 1988 JCB 107:1825), plays an important role in the organization of the postsynaptic apparatus at the neuromuscular junction.

532.10

DENERVATION OF THE NEUROMUSCULAR JUNCTION CAUSES A REDUCTION OF AGRIN-LIKE MOLECULES AND AChR-AGGREGATING ACTIVITY IN THE SYNAPTIC BASAL LAMINA. N. E. Reist, Dept. of Neurobiology, Stanford University, Stanford, CA 94305

The portion of a muscle fiber's basal lamina that lies in the synaptic cleft of the neuromuscular junction contains molecules that direct the aggregation of acetylcholine receptors (AChRs) and acetylcholinesterase (AChE) on regenerating myofibers. Agrin, a protein extracted from basal lamina-rich fractions of the electric organ of Torpedo, resembles these molecules in several ways. For example: 1) agrin directs the formation of AChR and AChE aggregates on cultured muscle fibers, 2) AChR-aggregating activity is present in skeletal muscle and can be immunoprecipitated by monoclonal antibodies against agrin, and 3) anti-agrin monoclonal antibodies stain molecules highly concentrated in the synaptic basal lamina. I have shown previously that denervation causes a reduction in the levels of anti-agrin staining at the neuromuscular junction. The study described here examined the possibility that denervation also causes a decrease in the synaptic basal lamina's AChR-aggregating activity, which might be expected if the active basal lamina molecules were molecules similar to agrin.

The cutaneous pectoris muscles of frogs were denervated for 4 weeks, at which time the muscles were damaged in such a way as to cause degeneration of all cellular elements at the neuromuscular junctions, while leaving the muscle fibers' basal lamina sheaths intact. New muscle fibers were allowed to regenerate within the basal lamina sheaths of the original muscle fibers for 3 weeks while reinnervation was prevented. Control muscles were not denervated prior to damage. The muscles were stained for AChE to localize the original synaptic sites on the basal lamina sheaths and AChRs were labelled with ^{125}I-α-bungarotoxin. The density of AChRs at former synaptic sites in the regenerated muscles that had been predenervated was 42.3% of control regenerated muscles ($p<<0.001$, student t-test). Thus denervation for 4 weeks caused a 2.4-fold reduction in the synaptic basal lamina's AChR-aggregating activity.

532.11

PEANUT AGGLUTININ-BINDING MOLECULES (PNA-BMs) COLOCALIZE WITH ACETYLCHOLINE RECEPTORS (AChRs) IN DEVELOPING NEUROMUSCULAR JUNCTIONS IN VITRO. L. Chen,*[1] C.-P. Ko[1] and I. Chow.[2] [1]Dept. Biol. Sci., Univ. Southern California, Los Angeles, CA 90089. [2]Dept. Biol., American Univ., Washington, D.C.20016.

To investigate the role of PNA-BMs in the formation of neuromuscular junctions (NMJs), we have examined PNA-BM and AChR distributions in muscle/nerve cultures of Xenopus laevis. PNA specifically recognizes glycoconjugate(s) in the extracellular matrix at NMJs in frog (Rana) (Ko, 1987) and in Xenopus tadpoles as shown by rhodamine-PNA and fluorescein- α-bungarotoxin staining. In culture, PNA-BMs begin to appear within 2 days of plating muscle cells and generally colocalize with AChR clusters. However, in 2-3 day cultures, AChR clusters outnumber and therefore are not always associated with PNA-BMs, whereas in older (5-6 days) cultures, PNA-BM distribution increases and colocalization with AChR clusters becomes more complete on both aneural and innervated muscle cells. Our results suggest that PNA-BMs appear after AChRs during synaptogenesis. How the distribution of basal lamina PNA-BMs might be regulated by nerve-muscle interaction during embryonic development is being investigated.

532.12

RELATIONSHIP OF INSULIN-LIKE GROWTH FACTOR I mRNA CONTENT TO SYNAPTOGENESIS IN RAT MUSCLE. G.W. Glazner* and D.N. Ishii (SPON: K.G. Beam) Physiology Dept., Colorado State Univ., Ft. Collins, CO 80523.

Insulin-like growth factor I (IGF-I) may play a role in the development of neural circuitry. For example, IGF-I stimulates neurite formation, and both it and its receptors are found in brain. The present study tested the hypothesis that IGF-I gene expression in muscle correlates with neuromuscular synaptogenesis in rats. IGF-I mRNAs were abundant about the time of birth, when polyneuronal innervation is maximal. These transcripts were down regulated with the same time course as postnatal elimination of excess synapses. Following nerve transection, adult muscle IGF-I mRNAs were up regulated, correlating with the renewed capacity of mature muscle to accept re-innervation when denervated. These data are consistent with a model in which (i) elevated IGF-I mRNAs support polyneuronal innervation, (ii) down regulation of IGF-I mRNAs contributes to loss of polyneuronal innervation, (iii) synaptogenesis provokes an inhibitory signal which is relieved upon nerve transection, and (iv) IGF-I follows its mRNA levels.

532.13

INFLUENCE OF HYDROCORTISONE (HC) ON ACETYLCHOLINESTERASE (AChE) AT THE NEUROMUSCULAR JUNCTIONS (NMJs) OF INNERVATED CULTURED HUMAN MUSCLE. V. Askanas, J. McFerrin*, C.S. Lee*, W.K. Engel. Neuromuscular Center, University of Southern California School of Medicine, LA, CA 90017.

In human muscle cultured in monolayer and innervated by fetal-rat spinal cord with dorsal root ganglia attached, AChE stain is present only at the NMJs (noninnervated cultured human muscle is negative in AChE stain). Under our standard conditions, all AChE sites are linear and thin between 5-21 days of innervation; between 4-8 wks of innervation, 37% of them become complexly organized. HC treatment, initiated after 4 wks of innervation and continued for 3 wks, strikingly increased complexity and intensity of AChE staining at the NMJs. In 5 experiments, 286 AChE sites (119 control, 167 HC-treated), were quantitated by computerized video-image analysis (RAS, Amersham). HC increased the size (in pixels) of AChE sites 2.7-fold and their optical density 1.1-fold (both $p<0.05$). Biochemically-measured total AChE activity increased 1.5-fold, and endplate-specific 16S fraction of AChE increased 2.7-fold ($p<0.001$). The influence of HC was related to its dose (maximum effect at 20 uM) and duration of treatment. Thus: 1) HC exerts significant influence on AChE at the NMJs; 2) increased staining intensity corresponds to increase of 16S-AChE; 3) the AChE increased by HC may play a role in the treatment of myasthenia gravis, in which both glucocorticoid and AChE inhibitors are commonly used.

532.14

CHARACTERIZATION OF SYNAPTIC VESICLE-ASSOCIATED PROTEINS IN NEUROBLASTOMA CELL LINES: EFFECTS OF DIFFERENTIATION AND OF CO-CULTURING WITH SKELETAL MUSCLE CELLS. **H.-Q. Han*, R.A. Nichols, M. Bähler* & P. Greengard. The Rockefeller University, New York, NY 10021.**

Synapsin I, synapsin II (Protein III), and p38 (synaptophysin) are synaptic vesicle-associated proteins, which may be involved in the regulation of neurotransmitter release. To characterize the levels and intracellular location of these proteins over the course of differentiation of neuroblastoma cell lines, biochemical and immunocytochemical assays were performed. Western blot analysis revealed basal levels of synapsin I, synapsin II, and p38 in extracts from the neuroblastoma cell lines NG108-15, NS26, NS20Y, NBr10A, NBr20A, and NCB20 (gifts from Dr. M. Nirenberg). Western blot and dot-blot immunobinding assays demonstrated that differentiation led to significant increases in the levels of synapsin I (3-fold), synapsin II (5-fold), and p38 (6-10-fold). Synapsin II exists as a doublet of synapsin IIa and synapsin IIb which are products of a single gene. Nevertheless, there was a preferential increase of synapsin IIa compared to synapsin IIb during differentiation of the neuroblastoma cells.

In double-immunofluorescent labeling of the neuroblastoma cultures using affinity-purified antibodies, the staining for synapsin I, synapsin II, and p38 was restricted to and co-localized in the trans-Golgi area. Following differentiation of the neuroblastoma cells, intense staining was found in neuritic varicosities, as well as in the trans-Golgi area. Staining for synapsin I, synapsin II, and p38 was also found to co-localize in neuritic varicosities. Co-culturing differentiated neuroblastoma cells with myotubes from fetal skeletal muscle led to an apparent enhancement of the staining for these proteins in neurites. After co-culturing for more than 5 days, these vesicle-associated proteins were found to be most highly concentrated at the contact sites between neuritic processes and myotubes, suggesting an accumulation of synaptic vesicles at potential synaptic sites.

TRANSPLANTATION: STRIATUM I

533.1

ELECTROPHYSIOLOGICAL AND MORPHOLOGICAL CHARACTERISTICS OF NEOSTRIATAL NEURONS TRANSPLANTED INTO THE SUBSTANTIA NIGRA OF THE RAT. S.M. Siviy, J.P. Walsh, M.S. Levine, C.D. Hull and N.A. Buchwald. MRRC, UCLA, Los Angeles, CA 90024.

Our laboratory has demonstrated functional synaptic connections between transplanted striatal neurons and host striatum (Synapse, 2:37). To further examine the extent to which grafts receive functional connections from the host, fetal neostriatal neurons were grafted into substantia nigra of adult rats that had received kainic acid lesions of the ipsilateral striatum 7-10 days before grafting. Two-5 weeks after transplantation, the rats were sacrificed, brains removed, and 400 μm thick slices through the transplant were taken for intracellular recording. To date we have recorded from 15 neurons. Grafted neurons had average resting membrane potential values of -46 ± 2.0 (±S.E.M.) mV. Input resistances (32.3 ± 3.5 MΩ) were higher than those of neostriatal neurons of the same age. Over half of the grafted neurons had received synaptic connections from the host, as indicated by the presence of excitatory post-synaptic potentials (EPSPs) in response to electrical stimulation of the host nigra. The EPSPs were generally of long duration (>100 msec) and often had multiple components. Recorded neurons labelled with Lucifer Yellow had morphological properties consistent with their age. Many of the neurons had dendrites that contained spines. Average diameter of somata was 17.1 ± 0.9 μm. This study shows that neostriatal neurons can be successfully grafted into the substantia nigra and form functional synaptic connections. Supported by USPHS Grant HD 5958.

533.2

TYROSINE HYDROXYLASE POSITIVE FIBERS IN TRANSPLANTED FETAL STRIATAL TISSUE: AFFERENT OR INTRINSIC? E.M. Zubrycki, M.E. Ragozzino, M. Giordano, M.T. Shipley and P.R. Sanberg. Depts. of Psychiat., Psychol. and Anatomy, Univ. of Cincinnati, Coll. of Med., Cincinnati, Ohio, 45267-0559.

The present study examined catecholaminergic afferents in long-term (7-8 month) fetal striatal transplants using tyrosine hydroxylase (TH) immunocytochemistry. Rats were bilaterally lesioned with quinolinic acid one month prior to receiving homotopic E17 striatal grafts. In approximately half of the transplants, TH positive fibers were found within the graft. Of interest, TH positive cell bodies were also found in some of the grafts. In order to find out where these cells may have originated, sagittal sections of E17 fetal brains were examined for TH immunocytochemistry. This revealed a small population of neurons positioned immediately ventral to the developing striatal ridge tissue. Specht et al, (J. Comp. Neurol., 199, 233-276, 1981) initially described these neurons as corresponding to the catecholaminergic groups A11-A14 in the adult rat brain. Further, they reported that immature versions of these neurons are present as early as E12.5. Having found catecholaminergic neurons in some of the transplants containing positive TH fibers, but not for every case, an obvious problem arises in regard to the origin of the fibers. The present findings point out the ease with which sampling contamination from catecholaminergic neurons can arise and suggests that caution be taken when interpretting the results of catecholaminergic immunocytochemistry in fetal striatal transplants.

533.3

DEVELOPMENT OF TYROSINE HYDROXYLASE (TH) POSITIVE NEURONS IN THE HUMAN EMBRYONIC SUBSTANTIA NIGRA (SN).
T.B Freeman, M.S. Spence*, and R. Miao*, Division of Neurological Surgery, University of South Florida, College of Medicine, Tampa, Fl. 33612 and Hana Biologics, Alameda, Ca. 94501.

There is widespread interest in the role of grafting human cadaver embryonic dopamine neurons into the striatum as a means of treating Parkinson's disease. In rodent models, the period of neuronal development at the time of donor tissue harvesting is critical. We have therefore examined the development of the human nigral dopamine system at post-conception (PC) 4 to 9.5 weeks.

TH-like immunoreactivity (TH-LI) was seen in the ventral mesencephalon by PC7. By PC 8 weeks there is an apparent increase in the number of TH-LI cells in the ventral mesencephalon. No TH-LI was observed prior to six weeks. The TH-LI cell bodies within the developing SN had very little cytoplasm and large nuclei, and first exhibited signs of neural process extension at PC 8 weeks. Prior to this time the cell bodies characteristically lacked processes. At PC 9 weeks, TH positive fibers were seen in the corpus striatum and in a region just anterior to the ventral mesencephalon.

In summary, the cells that form the presumptive nigrostriatal pathway in man become differentiated and begin extending axons from PC weeks 7-9.

533.4

FUNCTIONAL TRANSPLANTATION OF PROLIFERATED FETAL PIG DOPAMINE (DA) NEURONS IN THE PARKINSONIAN RAT MODEL.
B.D. Boss, R. Miao*, M.S. Spence*, D.H. Spector* and R.E. Strecker. Hana Biologics, Inc., Alameda, CA 94501 U.S.A.

We recently reported long-term survival and function of freshly dissected fetal pig CNS xenografts in a rat model of Parkinson's disease. Here we extend this finding by showing that grafts of cultured fetal pig DA neurons also function in this rat model. In addition, we have demonstrated that the DA-containing neurons are proliferating in our cell culture system. Donor tissue was dissected from the ventral mesencephalon of 21-24 day fetal pigs, enzymatically dissociated, mechanically triturated, and kept in proprietary media formulations for 15 days. Typically, 3 x 10^4 to 3 x 10^5 cells were plated per well; and, after 6-150x proliferation, the cells were transplanted (1 well/rat). The cultured tissue was injected directly into the DA- denervated striata of host rats. Eleven of 20 grafted rats have shown behavioral recovery in the amphetamine-induced rotation test. Preliminary histological analysis revealed very large grafts containing numerous DA neurons [as identified by tyrosine hydroxylase (TH) immunohistochemistry]. A parallel experiment combining immunohistochemistry and autoradiography has shown that when such cultures are labeled for 2 days in vitro with ^{3}H-TdR (days 5-7) numerous TH-positive grafted neurons containing label are found at 4 weeks post-grafting.

533.5

Detection of Tyrosine Hydroxylase mRNA in Transplanted Fetal Dopamineric Neurons.
Y. Solberg, Y. Pollack* and W. F. Silverman Units of Morphology (YS & WFS) and Immunology & Microbiology, Faculty of Health Sciences, Ben-Gurion University, Beer Sheva, Israel.

Tyrosine hydroxylase (TH) is the rate limiting enzyme in the synthesis of dopamine (DA). It is known that the gene, coding for production of this enzyme is strictly regulated, reflecting the functional state of the DA neuron. We have examined the expression of the TH gene using the *in situ* hybridization technique in transplanted fetal substantia nigra (SN) neurons as a way to address the question of functional integration into an adult host brain of grafted neurons. Solid tissue pieces were dissected from rat embryonic (E14) mesencephalon and were transplanted unilaterally into the striatum of normal, adult male Sprague-Dawley rats. As a control, some rats were implanted with non-DA embryonic tissue. Sections from adult mesencephalon, containing substantia nigra served as a positive control. A month after transplantation, the rats were sacrificed, and their brains processed for *in situ* hybridization using a specific ^{35}S-labelled probe for TH mRNA (generously provided by D. Chikaraishi). Many cells, positive for TH mRNA were distributed heterogeneously within the transplants. In contrast, cells in the host striatum, and in non-DA grafts, did not exhibit a positive signal. As expected, numerous cells positive for TH mRNA were present in the adult SN. We believe this work represents an important new approach to the study of graft cell function in a host brain.
Supported by the Foulkes Foundation (YS), Brookdale Institute for Gerontology (YS) and Israel Institute for Psychobiology (WFS).

533.6

CHRONIC L-DOPA TREATMENT DECREASES THE VIABILITY OF GRAFTED AND CULTURED EMBRYONIC RAT MESENCEPHALIC DOPAMINE NEURONS.
K. Steece-Collier, T.J. Collier, C.D. Sladek and J.R. Sladek Jr. Depts. Neurobiology and Anatomy and Neurology, University of Rochester School of Medicine, Rochester, NY 14642.

Neural transplantation of dopamine (DA)-secreting cells (embryonic substantia nigra neurons; adrenal medulla) is being studied as an experimental therapy for Parkinson's disease. Most of these patients require pharmacological treatment with L-DOPA to control behavioral symptoms. However, it is not known what effect this drug may have on the viability of grafted tissue. To examine this important aspect of transplantation therapy we are examining the effect of chronic L-DOPA treatment on cultured and grafted embryonic rat mesencephalic neurons. In monolayer cultures of day E13-16 mesencephalic cells, L-DOPA caused a dose dependent decrease in the number of tyrosine-hydroxylase (TH)-positive neurons (-60% with 10^{-4}M L-DOPA). Furthermore, TH-positive neurons treated with all concentrations of L-DOPA (10^{-8}M-10^{-2}M) exhibited fewer branched neurites and decreased neurite length compared to untreated controls. In rats hosting fetal DA neuron grafts in the DA-depleted caudate nucleus, chronic Sinemet treatment (32mg/kg, i.p., twice daily for 6 weeks) yielded a marked decrease in TH immunoreactivity of grafted neurons, and a marked increase in macrophage activity within grafts. These preliminary data suggest that continued L-DOPA treatment in Parkinson's disease patients receiving neural grafts may have adverse effects on survival and growth of transplanted DA neurons.
Supported by AG00847, NS08423 and the PEW Foundation.

533.7

ENHANCED SURVIVAL AND NEURITE EXTENSION FROM EMBRYONIC RAT DOPAMINE NEURONS CO-CULTURED WITH ADULT RAT SCIATIC NERVE: A DIFFUSIBLE FACTOR OTHER THAN NERVE GROWTH FACTOR. IMPLICATIONS FOR NEURAL GRAFTING.
T.J. Collier, J.E. Springer, M.J. Gallagher, C.D. Sladek and J.R. Sladek Jr. Depts. Neurobiology and Anatomy and Neurology, University of Rochester School of Medicine, Rochester, NY 14642 and Hahnemann University School of Medicine, Philadelphia, PA 19102.

We have been investigating methods for enhancing embryonic dopamine (DA) neuron viability and plasticity as an adjunct to our ongoing studies of DA neuron grafts in experimental models of Parkinson's disease in rats and non-human primates. These studies include initial screening in monolayer cultures of fetal rat ventral mesencephalon, followed by co-grafting experiments in unilaterally DA depleted rats. We have found that a factor derived from segments of adult rat sciatic nerve significantly enhances the viability of developing midbrain DA neurons. Cultures consisted of cell suspensions derived from day 13-16 embryonic F344 rats plated at a density of 500,000 cells/16mm diameter plastic well. Cultures of midbrain cells were switched to low (1%) serum medium, or serum-free medium, 72 hours after plating, and baskets containing a 10mm segment of adult rat sciatic nerve were added to experimental wells. In this arrangement, nerve segments and embryonic neurons shared medium but were not in physical contact. Cultures were fixed and stained 7-10 days later for tyrosine-hydroxylase (TH) as a marker for DA neurons. TH-positive neurons co-cultured with sciatic nerve exhibited enhanced survival (1.5-8X) and enhanced neurite outgrowth. This effect was reproduced by conditioned medium from co-cultures, and was not prevented by addition of antibodies to laminin (1:200) or nerve growth factor (NGF, 1:100). In a second experiment, we implanted fetal DA neurons into the denervated caudate nucleus of rats with unilateral lesions of the nigrostriatal DA pathway, with or without a co-graft of sciatic nerve placed into the ipsilateral lateral ventricle. Tests of amphetamine-induced rotational behavior in these animals suggests that co-grafted subjects normalize behavior approximately 4 weeks after grafting, while animals hosting DA grafts alone require 5-6 weeks to recover. Anatomical correlates of DA grafted and co-grafted animals will be presented. These findings suggest that a diffusible factor, other than NGF or laminin, derived from adult rat peripheral nerve enhances viability of developing DA neurons in culture and neural grafts.
Supported by the PEW Foundation and the Alzheimer's Association (ADRDA).

533.8

FETAL STRIATAL TISSUE TRANSPLANTS INTO NORMAL STRIATUM: EFFECTS OF VARIOUS TISSUE TYPES ON BEHAVIOR.
S.Y. Lu, K. Bertram, A.B. Norman and P.R. Sanberg. Div. Neurosci., Depts. Physiol.& Psychiat., U. of Cincinnati, OH 45267

We previously found that abnormal behavior can result from the transplantation of fetal striatal brain tissue into the normal rat. The present study examined the effects on locomotor behavior of transplants of different fetal brain areas on locomotor behavior. Male Sragues-Dawley rats (n=27) received bilateral transplants of either E14-15 fetal cortex, substantia nigra or striatum into the striatum. Another group of rats (n=9) received adrenal medulla from 3 week old rat pups. A sham control group received vehicle only. A normal control group consisted of unoperated rats. Spontaneous nocturnal locomotion was recorded in Digiscan Activity Monitors 1 week before and 5 weeks after transplantation.

During the first nocturnal hour, the cortex transplant group were significantly hyperactive compared to both control groups in vertical activity, vertical time measurements, and also in horizontal activity and stereotypic activity; in contrast the substantia nigra group were hypoactive in horizontal activity and stereotypic activity. The groups receiving striatum or adrenal medulla did not show significant differences from the control groups.

These preliminary results indicated that transplants of fetal brain tissue into the intact striatum caused perturbation of noctural locomotion. Furthermore, transplants of different fetal brain areas produced different locomotor changes. Whether these abnormal locomotor behaviors induced by transplants are due to physical or neurochemical characteristics of the transplants is under investigation.

533.9

FETAL STRIATAL TRANSPLANTS PROTECT AGAINST QUINOLINIC ACID LESIONS IN THE STRIATUM: A BEHAVIORAL ANALYSIS. M. Levivier*, S.H. Pearlman, T.J. Collier, J.R. Sladek, Jr., and D.M. Gash. Neurobiology and Anatomy, University of Rochester, Rochester, NY 14624, USA, & *Department of Neurosurgery, Erasme Hospital, Universite Libre de Bruxelles, Brussels 1070, Belgium.

Previous studies have reported that transplants of neonatal striatum exert a protective effect on the host when simultaneously injected with one of the excitatory neurotoxins kainic acid or quinolinic acid (QA). The present experiment further supports these studies by demonstrating that fetal striatal transplants protect the striatum against QA insult as measured by apomorphine induced rotational behavior.

Adult female Long Evans rats (n=32) received two unilateral intrastriatal transplants (2 µl/ site). Tissues grafted included fetal striatum, neonatal adrenal medulla, adult sciatic nerve, adipose tissue or sham surgery. One week after implants, rats were unilaterally injected with 240 nmol QA (1.0 µl) at an injection site midway between the two implants. Apomorphine (0.5 mg/kg, s.c.) induced rotational behavior was measured three times during the study: pre-graft, one week post-graft, and 1 week post-QA lesion. All groups of animals exhibited equivalent modest rotation (12-40 turns/60 mn) away from the side of implants during baseline and post-implant tests. After QA lesions, all animals except those receiving fetal striatal implants, exhibited a reversal of rotation direction, now turning toward the side of implants and lesion presumably reflecting an imbalance due to striatal damage by QA. Subjects hosting striatal grafts maintained their pre-lesion behavior, suggesting protection for the QA lesion. Anatomical analyses of grafts and lesions will be discussed. These results suggest that fetal striatal implant also provide a protective influence against toxic QA insult, and this protection can be measured using behavioral paradigms. (Supported by the PEW Neuroscience Initiative)

533.10

BILATERAL NIGRAL GRAFTS TO THE STRIATUM OF WEAVER MUTANT MICE ENHANCE LOCOMOTOR COORDINATION W.C. Low, L.C. Triarhou, Y. Kaseda, J. Norton*, and B. Ghetti. Depts of Physiology and Biophysics, Pathology (Neuropathology), Psychiatry, Medical Genetics, and Program in Medical Neurobiology, Indiana University School of Medicine, Indianapolis, IN 46223

The behavioral effects of dopamine-rich intrastriatal nigral grafts have been extensively examined in rodents using the Ungerstedt model of rotational assymetry after chemical lesions of the nigrostriatal pathway. The focus of the present study was to examine the effects of intrastriatal nigral grafts on locomotor coordination in weaver mutant mice, a genetic model of nigrostriatal dopamine deficiency. Behaviorally these mutants exhibit an instability in locomotor coordination characterized by frequent tumbling while walking. Locomotor coordination was examined by quantifying tumbling activity in an open field during a 5 minute period of time. Wild type mice placed in an open field did not tumble during the period of observation. Weaver mutants, on the other hand, fell 14.0 ± 4.6 times (mean ± SEM). Mutants with unilateral and bilateral cortical lesions exhibited similar tumbling activity and fell 13.0 ± 3.4 and 16.8 ± 8.3 times respectively. Weaver mutants with unilateral grafts showed no improvement in locomotor coordination and fell 22.8 ± 6.8 times. In contrast, weaver mutants with bilateral solid grafts displayed a reduction in locomotor tumbling and fell only 4.3 ± 1.4 times. Paired comparisons of animals with bilateral grafts before and after transplantation revealed a significant reduction in tumbling activity after grafting ($P < 0.05$). Statistical comparisons between the different groups using log transformed data revealed that tumbling activity by animals with bilateral grafts was significantly less than mutants with unilateral grafts, and mutants with unilateral and bilateral lesions ($P < 0.05$), and not significantly different from wild type mice. Animals with unilateral grafts, on the other hand, were significantly different from wild type mice ($P < 0.05$), but not significantly different from weaver mutants, mutants with unilateral lesions, or mutants with bilateral grafts. These results suggest that bilateral grafts can significantly reduce tumbling activity in weaver mutant mice which unilateral grafts alone are not capable of accomplishing. (Supported by RO1-NS-14426)

533.11

DECREASE OF LESION-INDUCED DOPAMINERGIC SUPERSENSITIVITY IN THE RAT BY FETAL NIGRAL GRAFTS. L. Rioux, D. Gaudin*, P.J. Bédard, L. Grégoire*, C. Gagnon* and T. Di Paolo. Lab. Neurobiol., Hôp. Enfant-Jésus and Dept d'Endocrinol. mol., Laval University Hospital Center, Québec, (QC), and School of Pharmacy, Laval University, Ste-Foy, (QC), Canada.

Female rats were lesioned with 6-OHDA 8µg in 2µl in the left substantia nigra. At least one month later they were tested with amphetamine 5 mg/kg s.c. and apomorphine 0.35 mg/kg s.c. A suspension containing approximately 1.5 x 10^6 cells from the ventral mesencephalon of rat embryos was distributed in three sites in a triangular fashion in the center of the denervated striatum. Grafted DA neurons reinnervated the medial part of dorsal striatum and reversed the rotational assymetry evoked by amphetamine. Apomorphine, 0.25 mg/kg, given 4 to 6 months post-graft still elicited contraversive circling but the number of turns was reduced. Circling evoked by CY208243 (Sandoz), 0.5 mg/kg (D1 agonist) s.c. or LY171555 (Lilly) 0.5 mg/kg I.P., (D2 agonist) was respectively 40% and 37% less in grafted rats than in lesioned non grafted rats. The distribution and density of dopaminergic receptors in the striatum of grafted and lesioned rats were determined by autoradiography by means of *in vitro* binding with [^{3}H]SCH-23390 for D1 receptors and [^{3}H]-spiperone for D2 receptors. Preliminary results show that supersensitivity of D2 receptors almost disappeared in the medial part of dorsal and ventral striatum of grafted rats while supersensitivity of D1 receptors only decreased in the medial part of dorsal striatum. This study suggests that functional recovery following nigral grafts could involve DA receptors regulation. Supported by MRC of Canada.

533.12

DEFICITS IN DISENGAGE BEHAVIOR ARE NOT AMELIORATED BY INTRASTRIATAL MESENCEPHALIC GRAFTS THAT REVERSE AMPHETAMINE-INDUCED ROTATIONAL BEHAVIOR. R.J. MANDEL, A. NORRMAN*, C. HAAPANIEMI*, and P. BRUNDIN*, A. BJÖRKLUND. University of Lund, Dept. Med. Cell Res., Biskopsgatan 5, S-223 62 Lund Sweden.

Schallert and Hall (*Behav. Br. Res.* 30:15-24, 1988) have reported that rats with unilateral 6-hydroxydopamine (6-OHDA) lesions of the nigrostriatal tract that have recovered their orienting response to perioral tactile stimulation contralateral to their lesion do not respond to the identical perioral stimulation while eating. This study examined the effects of intrastriatal DA-rich fetal suspensions of ventral mesencephalon (VM) on rotational behavior, sensory inattention, and disengage behavior of animals with unilateral dopamine depletions of striatum. Fifteen rats received unilateral 6-OHDA depletions of striatal DA, were tested for rotation, and then received either, 1) suspensions of fetal VM in the central striatum, 2) suspensions of fetal VM in striatum and a radiofrequency lesion (RF) of striatal efferents, or 3) no treatment. Six weeks after grafting the rats were tested for disengage behavior for 5 days, followed immediately by 1 test for sensory inattention (perioral stimulation in the absence of food), and again for rotational behavior. All rats in the VM grafted group reversed their 6-week post-grafting rotation while no rats in either the RF lesioned or 6-OHDA only groups changed their levels of rotation. On the other hand, there were no group differences measured on either disengage behavior or sensory inattention. While eating, all animals displayed a robust response to the tactile probe ipsilateral to the lesion but almost never responded to stimulation on the contralateral side. While some rats still displayed sensory inattention contralateral to the lesion, other rats oriented to the stimuli in the contralateral hemifield. Thus, grafted rats that had reversed their rotational behavior and could orient to stimuli contralateral to the lesion, nevertheless did not orient to contralateral stimulation while eating. Studies utilizing intrastriatal VM graft placements known to ameliorate sensory inattention deficits but not rotational behavior are underway to test their effect on disengage behavior.

533.13

REPEATED ADMINISTRATION OF L-DOPA PLUS BENZERAZIDE IN 6-OHDA LESIONED FEMALE RATS IN PRESENCE OR ABSENCE OF FETAL DOPAMINE NEURONS GRAFTED INTO THE STRIATUM. D. Gaudin*, L. Rioux, P.J. Bédard, L. Grégoire*, C. Gagnon* and T. Di Paolo. (SPON: R. Boucher). Lab. Neurobiol., Dept. Anatomy and Dept Mol. Endocrinol., Univ. Laval, Québec, (QC), Canada G1K 7P4.

We have previously shown (Rouillard, C. Neuropharmacology, 26: 1601, 1987) that repeated administration of L-DOPA in rats bearing a unilateral lesion of the substantia nigra by 6-OHDA causes a progressive increase in the circling response and a further increase in [^{3}H] spiperone binding on the lesioned side. In the present study we wanted to investigate whether a graft of fetal dopaminergic neurons would prevent both the behavioral and biochemical supersensitivity. In a group of female rats, we performed a unilateral nigral lesion with 6-OHDA. The animals were then tested for circling with amphetamine 5 mg/kg and apomorphine 0.35 mg/kg. The animals which displayed circling with these two drugs were then divided into two groups. One group received a graft of 1.5 x 10^6 cells taken from the ventro-mesencephalon of 13-14 day old rat embryos. The other group was not grafted. 4 to 6 months after the graft, all animals received repeated injections of L-DOPA + benzerazide. Each group of animal received of 1,2,8 or 14 injections of L-DOPA (100 mg/kg i.p.) plus benzerazide (50 mg/kg i.p. 1/2 hour before) separated by 24 hours. Circling was monitored during five hours after 1,2,8 and 14 injections. Seventy-two hours after the circling test, the animals were sacrificed by decapitation and the striata rapidly removed for subsequent binding studies. The group without graft showed a significant increase of the circling response (194% and 205% respectively for L-DOPAx8 and L-DOPAx14), while the group with graft showed a significant increase of 177% only for L-DOPAx14. In the group without graft, L-DOPAx2 showed a small, non significant increase of the circling response, whereas no increase for L-DOPAx8. Determination of the density of dopaminergic receptors is still in progress and should allow a better comprehension. This study suggests that nigral grafts produce a delay of the appearance of the supersensitivity induced by repeated administration of L-DOPA. (Sup. MRC of Canada).

533.14

ELECTROPHYSIOLOGICAL AND BIOCHEMICAL STUDIES ON HUMAN FETAL MESENCEPHALIC GRAFTS TO DOPAMINE DENERVATED STRIATUM IN IMMUNOSUPPRESSED RATS. I. Strömberg*, C. van Horne, J. Masserano, T. Mahalik, M. Bygdeman*, Å. Seiger*, N. Weiner, L. Olson*, and B. Hoffer. *Karolinska Inst., Stockholm, Sweden; U Colo. Med. Ctr., Denver, CO 80262.

Human fetal mesencephalic tissue, obtained following termination of first trimester pregnancies, was grafted to the ventricle of previously unilaterally DA-denervated rats. Apomorphine-induced rotations before grafting showed a double peak pattern. After grafting, significant reductions in rotations were seen for up to 6 months. Extracellular recordings from the striatal cells showed a low firing rate (1.8±0.4 Hz, n=25) on the grafted side, suggesting that the striatal cells were reinnervated by DA containing terminals. On the intact contralateral side, the firing rate was 1.2±0.4 (n=8). Cis flupenthixol, a DA-receptor antagonist locally applied to striatal cells ipsilateral to the graft, increased the firing rate, probably by blocking the effect of DA released from graft-derived terminals. Electrophysiological recordings within the graft indicated the existence of dopaminergic cells with initially positive action potentials of long duration (2.0-5.0 ms). DA levels were approximately 5 times higher in the grafts as compared with the intact substantia nigra. Fractional release from striatal minces after K^+ stimulation was similar for grafted and control sides and was inhibited by the D2-receptor agonist quinpirole. TH-immunoreactive nerve terminals, innervating the striatum, originated from the graft which had many TH-positive cells. Taken together, these data show that human fetal dopaminergic tissue reinnervates denervated striatum and reduces apomorphine induced rotation. Electrophysiologically active dopaminergic cells and high DA levels are found within the graft.

533.15

SOLUBLE NEURITE PROMOTING ACTIVITY IN FETAL OR TERM AMNION M.A. PALMATIER, R.J. PLUNKETT* and I.J. KOPIN CNB and SNB, NINDS, NIH, Bethesda, Maryland 20892

The basement membrane of full term human amnion has been shown to be a permissive substrate for neurite outgrowth in vitro and in vivo. We have investigated fetal and term amnion as a source of soluble neurite promoting activity for use in animal models of Parkinson's Disease. Monkey and human fetal amnion and human term amnion was tested for soluble neurite promoting activity by incubating amnion with dorsal root ganglion (DRG) explants in serum-free medium. Neurite outgrowth from the DRGs was compared to neurite outgrowth from DRGs induced by various concentrations of NGF. All amnion tested induced neurite outgrowth from DRG explants. The neurite outgrowth is not blocked by antibodies to mouse NGF. Neurite outgrowth from DRG explants did not require contact with the amnion. During long-term storage of amnion in phosphate-buffered saline (PBS) at 4°C there is loss of neurite promoting activity from the amnion and appearance of neurite promoting activity in the PBS. These results suggest that amnion contains a soluble neurite promoting activity.

TRANSPLANTATION: STRIATUM II

534.1

SOLID ADRENAL GRAFTS IN LONG-TAILED MACAQUES: STEREOTAXIC IMPLANTATION AND BIOCHEMICAL STIMULATION M. Dubach & D.C. German, Psychiatry and Behavioral Sciences and Regional Primate Research Center, University of Washington. Seattle WA; and Departments of Physiology and Psychiatry, University of Texas, Dallas TX.

Tissue-culture experiments have demonstrated the ability of biochemicals such as nerve growth factor and laminin to increase the survival and neurite production of adrenal medulla cells, depending on donor age and species. Here we present efforts to establish a reliable method for CNS transplantation of adrenal medulla in long-tailed macaques by including these substances in the medium during transplantation. In some cases grafts were further supplemented by chronic infusion of nerve growth factor into brain tissue or lateral ventricle.

The physical preparation of graft tissue may also be critical for viability. Previous solid grafts have involved diced tissue preparations, but in the present experiments we have grafted intact strips. The medulla as a whole was separated from surrounding cortex and sliced into strips of equal thickness. Each strip was immersed in HBSS with or without biochemical supplement, loaded with its medium into a 19ga spinal needle, and stereotaxically implanted into dopamine-denervated caudate or putamen. Turning behavior of the unilaterally treated animals was monitored by rotometer. After 3-4 weeks, graft and brain tissue were examined by tyrosine hydroxylase immunohistochemistry. (Supported by USPHS grants NS25724, RR00166, and Dallas Area Parkinsonism Soc.).

534.2

BEHAVIOR REVERSAL IN RAT MODELS OF PARKINSON'S DISEASE FOLLOWING GRAFTING OF FREEZING AND THAWING ADRENAL MEDULLARY TISSUE. G.F. Zhang*, Q. Lu*, Y.S. Wang* and S.S. Jiao. Beijing Institute for Neuroscience, Capital Institute of Medicine, Beijing, 100054, China

In this study the feasibility of storing adrenal medulla at low temperature was investigated by observing the morphology of graft cells and testing its function. The medulla grafts taken from adult Sprague-Dawley rats, frozen at the rate of decreasing 1°C per minute, stored at -196°C for one week, were transplanted into the caudate nucleus of host brain, where the unilateral substantia nigra was damaged by 6-OHDA. During the observation period of three months, the test for rotation behavior induced by apomorphine shows that the rotation reduced partly after operation, and that the longer the rat survived, the less the number of rotation reduced. Histological examination reveals that the shape of graft cells and the feature of staining are normal, and that abnormal tissue reaction and degeneration do not occur in host brain. Histofluorescent study shows the graft cells still maintain the function of secreting catecholamine. It is suggested that freezing to low temperature does not adversely affect the ability of adrenal medulla tissue to survive, and that by the low temperature frozen-storage method, the medulla grafts may be utilized for experimental research and clinical application. (Supported by NHRF 86-230)

534.3

TRANSPLANTATION OF CULTURED HUMAN FETAL ADRENAL GLAND-DERIVED CELLS INTO RODENT BRAIN. R.E. Strecker, J.F. Loring, T.K. Huffaker*, M.S. Spence*, C.L. Spurgeon*, D.R. Elliott*, T.F. Pfankuch*, E.G. Goldbach*, W.A. Neal*, R. Miao, A.S. Morgan*. Hana Biologics, Alameda, CA. 94501

Reports of adrenal autografts as a therapy for Parkinson's disease (PD) stimulated our interest in combining tissue culture and grafting of human fetal adrenal, thereby eliminating the use of the patient's own adrenal for grafting. Adrenal medullary precursor cells are of neural crest origin and can be induced to express a catecholamine neuronal phenotype in culture. First, we histologically characterized human fetal adrenals and identified 3 cell types, one of which fit our criteria for the neural precursor cell type. Next, dissociated whole adrenals were cultured in proprietary media formulations resulting in cultures with high viability, large numbers of tyrosine hydroxylase positive (TH+) cells, and containing Norepi, Epi and DA, as measured by HPLC. Finally, nude mice and immunosuppressed PD rats received grafts of cultured tissue, freshly prepared cell suspensions, or tissue pieces. Several co-culturing/co-grafting conditions were also tested (glia, Sciatic nerve, NGF). Low numbers of TH+ neuron-like cells (<50) were seen in some cases, but most grafts appeared TH negative, even as soon as 1 week post-grafting. Our results support the possibility that the therapeutic effects of the adrenal autograft procedure may not be mediated by catecholamine cells.

534.4

THE EFFECT OF ADRENAL MEDULLARY GRAFTS IN MPTP TREATED AGING MOUSE BRAIN. I.Date,S.Y.Felten,D.L.Felten. Dept. Neurobiol.& Anatomy,Univ. of Rochester Sch. of Med., Rochester, NY 14642

We have investigated the effect of systemic injection of MPTP and subsequent grafts of adrenal medulla into the striatum of young(2-3mo.) and aging(12mo.)C57Bl/6 mice.

MPTP treatment (4X20 mg/kg i.p. given 3 or 12 hr apart) resulted in 80-90% depletion of striatal dopamine(DA) and disappearance of tyrosine hydroxylase(TH)-immunoreactive(IR) fibers in both young and aging mice 1 week following treatment. Although partial recovery of DA content and TH-IR fibers was observed in young mice 5 weeks after treatment, aging mice showed no apparent recovery. Adrenal medullary minced pieces were grafted into the striatum of MPTP-treated young and aging mice. In young mice, dense TH-IR fibers were observed in the grafted striatum and the DA content on the graft side recovered to 45% of the normal level. In aging mice receiving similar grafts, TH-IR fibers also were observed in the grafted striatum, but less dense and more restricted around the site of the graft compared with young mice. DA content on the grafted side was 29% of the normal level. These observations indicate that the nigrostriatal DA system in aging brain could be recovered by adrenal medullary grafts, but the degree is more limited compared with young brain. Supported by NIH R37 AG06060 and a PEW Foundation Center Grant.

534.5

DECREASED ASYMMETRY IN STRIATAL EXTRACELLULAR DOPAMINE WITH BEHAVIORALLY EFFECTIVE ADRENAL MEDULLA GRAFTS. Jill B. Becker and William J. Freed. Univ. of Michigan, Neuroscience Laboratory Bldg, Ann Arbor, MI 48104-1687 & NIMH, St. Elizabeth's Hospital, Washington, D.C.

Adrenal medulla grafts in the lateral ventricle reduce the behavioral manifestations of striatal dopamine (DA) depletion in an animal model of Parkinson's Disease. Results from this lab have demonstrated that adrenal medulla grafts increase brain DA turnover, and amphetamine-stimulated striatal DA release without producing an increase in CSF DA or basal extracellular concentrations of DA in striatum. The experiments reported here were undertaken to further define the mechanism(s) through which adrenal medulla grafts mediate recovery of function.

Adult male rats with unilateral 6-OHDA lesions of the substantia nigra and intraventricular adrenal medulla grafts underwent in vivo microdialysis 5 months post-graft. Dialysis probes were placed into both the DA-denervated and intact striata. Basal concentrations of DA, DOPAC and HVA were determined 24 later in freely moving animals.

Graft-induced behavioral recovery (> 10% decrease in APO-induced turning) was associated with decreased asymmetry in basal extracellular DA in striatum ($p < 0.02$; compared to animals with adrenal medulla grafts that did not decrease turning). The difference in asymmetry was due to decreased extracellular DA on the intact side rather than increased DA on the graft side. DOPAC and HVA concentrations were also decreased on the intact side (compared with control animals). Therefore, recovery of the response to APO may be associated with graft-induced compensatory changes in striatal DA activity of the contralateral striatum. (Supported by NIH grants #NS22157 & #NS01056).

534.6

INCREASED BLOOD-BRAIN BARRIER PERMEABILITY ASSOCIATED WITH ADRENAL MEDULLA GRAFTS. E.J. Curran and J.B. Becker. Neuroscience Program and Department of Psychology, The University of Michigan, Ann Arbor, MI 48109.

In rats with unilateral 6-OHDA lesions of the substantia nigra (SN), intraventricular adrenal medulla grafts reduce amphetamine (AMPH)- and apomorphine (APO)-induced rotational behavior, but the mechanism involved in this behavioral recovery is unknown. We hypothesize that increased permeability of the blood-brain barrier (BBB) adjacent to the graft allows dopamine (DA) access to the denervated striatum.

Adult rats with unilateral SN lesions were tested for APO- and AMPH-induced rotational behavior prior to and after receiving intraventricular adrenal medulla grafts or control tissue. Animals were then tested using *in vivo* microdialysis. Some grafts resulted in a decreased behavioral response to AMPH while others produced a decrease in the response to APO. Animals that showed a decreased behavioral response to AMPH had AMPH-induced DA release in the striatum that was seen using microdialysis; when DA was injected into the jugular, penetration into the denervated striatum occurred with the average increase in striatal DA being from 9.45 pg/ 5 ul to 91.45 pg/ 5ul. However, animals that showed a decreased behavioral response to APO and control animals, did not show either the AMPH-induced DA release or the penetration of peripheral DA into the denervated striatum. These results show that: (1) an increase in BBB permeability is associated with AMPH-induced DA release and with a decreased behavioral response to AMPH, and (2) there may be more than one mechanism involved in mediating the different behavioral effects of adrenal medulla grafts. (Supported by NS22157).

534.7

QUANTITATIVE ASSESSMENT OF CO-GRAFTING OF ADRENAL MEDULLARY TISSUE AND SURAL NERVE IN MPTP PARKINSONISM. E. Worthy*, R.L. Watts, A.S. Mandir, P.M. Iuvone, C. Herring, R.A.E. Bakay (SPON: J. Juncos). Depts. of Neurology, Neurosurgery, & Pharmacology, Emory Univ. & Yerkes Primate Research Center, Atlanta, GA 30322.

We have employed a learned, visually-instructed right wrist flexion/extension motor task to study co-grafting of autologous adrenal medullary tissue and sural nerve into the left caudate nucleus in 2 MPTP hemiparkinsonian macaques. In this stimulus-initiated task, reaction time is measured from a "GO" signal to movement onset (RT/MO) and movement time (MT), the physiological correlate of bradykinesia, is measured from movement onset to achievement of final target. Right hemiparkinsonism induced by left intracarotid injection of .6 mg/kg of MPTP-HCl resulted in a prolongation of movement time and reaction time in both animals: Animal #1 - RT/MO 300 ± 10 [mean ± SEM in msec] and MT 110 ± 35 in normal state vs. RT/MO 519 ± 49 and MT 314 ± 24 eight months after MPTP; Animal #2 - RT/MO 210 ± 13 and MT 160 ± 12 in normal state vs. RT/MO 582 ± 72 and MT 2058 ± 73 one year after MPTP. Co-grafting was performed via a transcortical intraventricular approach with direct implantation of adrenal medullary and sural nerve tissue fragments into the head of the left caudate nucleus. Preliminary results from animal #1 at 5-6 months after co-grafting reveal an improvement of MT (147 ± 33) and RT/MO (423 ± 29) compared to the parkinsonian state.

534.8

DOPAMINE POLYMER RELEASE REDUCES ROTATIONAL ASYMMETRY IN 6-OHDA LESIONED RATS. S.R. Winn, L. Wahlberg *, P.A. Tresco *, A. Signore *, P. Aebischer. Artificial Organ Laboratory, Brown University, Providence, RI 02912.

The behavioral effect of sustained intrastriatal release of DA from a polymer matrix was analyzed in a 6-OHDA unilaterally lesioned rat model. Rods of poly[ethylene-vinyl acetate copolymer] (EVAc) containing 20% by weight of dopamine were pressure extruded and coated with several layers of pure EVAc. Biocompatible semipermeable acrylic copolymer receptacles were implanted stereotaxically in the striata of lesioned rats. Five animals received dopamine loaded rods, while controls received rods comprised of EVAc alone. Two weeks postimplantation, experimental animals showed an 85% improvement in their rotational behavior under apomorphine challenge. Two weeks after the removal of the dopamine-releasing rod, rotational behavior increased again, leaving no statistical difference between the control and experimental group 4 weeks postremoval. In vivo microdialysis experiments were performed in 6 animals which had received DA-releasing EVAc rods: 3 acutely and 3 after 7 days. Twenty min after the implantation of a 20% DA-releasing EVAc polymer rod, detectable levels of DA were recovered. The DA levels remained elevated throughout the next 180 min. Significant extracellular striatal DA was present in the lesioned striatum 7 days postimplantation of DA/EVAc rods. Sustained release of dopamine from a polymeric matrix placed within a semipermeable receptacle alleviates experimental parkinsonism in rats. This technique offers the advantage of direct target access while preventing subsequent damage during placement or retrieval of loaded polymer matrices.

534.9

IMPLANTABLE MICROENCAPSULATED DOPAMINE (DA) : A COMPARISON OF PROLONGED DA RELEASE FROM TWO DIFFERENT POLYMER EXCIPIENTS. A.McRae, S.Hjorth, D. Mason*, L. Dillon* & T. Tice*. Dept of Histology & Pharmacology Univ of Goteborg Goteborg, Sweden 40033 Southern Research Inst. B'ham, Al 35255

A major advantage of employing poly [DL- lactide co-glycolide] for biodegradable controlled-release microcapsule systems within the CNS is the ability to modify the duration of drug release by manipulating the biodegradation kinetics of the polyester i.e. changing the ratio of lactide and glycolide in the copolymer. DA was encapsulated in 2 different excipients. One is a 50:50 lactide/ glycolide coplomyer (DA 50:50); the other is a DA 65:35 lactide / glycolide coplomyer (DA 65:35). The 65:35 copolymer biodegrades slower than the 50:50 copolymer thus, allowing a longer release of DA. Male rats (Sprague Dawley) were unilaterally lesioned under barbital anesthesia in the MFB using 6-OHDA (8µg/4µl). When a stable baseline apomorphine rotational response was established rats were stereotaxically injected under ether anaesthesia with 3 µl of a DA microcapsule supension of either DA 50:50 or DA 65:35 in 2 sites in the denervated striatum.Thirty minutes following implantation rotational behavior was recorded for 2-3 hours. Implantation of DA 50:50 microcapsules elicited immediate contralateral rotation whereas there was a 60-90 minute delay of contralateral rotation following injection of DA 65:35. Apomorphine induced rotation was decreased by 50% up to 8 weeks in rats bearing DA 65:35 microcapsules. Implanted DA 50:50 microcapsules did not attenuate apomorphine induced rotational behavior. These results show that by modifying the polymer excipient it is possible to attain functionally significant amounts of DA in the CNS for prolonged periods of time by single administration of microencapsulated DA.

534.10

RETROVIRAL-MEDIATED GENE TRANSFER AND EXPRESSION OF ACTIVE HUMAN TYROSINE HYDROXYLASE IN TRANSPLANTABLE CELL LINES. EI Ginns*, SL Cottingham, M Schultzberg, BM Martin,* ME LaMarca*, and SM Paul*. NSB, NIMH, Bethesda, MD 20892 (Sponsor: FK Goodwin).

Tyrosine hydroxylase (TH) catalyzes the rate-limiting step in the synthesis of catecholamines and has been implicated in several human neuropsychiatric disorders. In order to produce defined clonal transplantable cells, the retroviral vector pDOLMP10 containing cDNA for form 2 of human tyrosine hydroxylase (HTH-2) was used to transfer human TH activity to NIH-3T3 cells. TH enzyme activity was examined in G418-resistant transfected 3T3 fibroblasts and enzyme kinetics of HTH-2 were characterized.

Northern blot analysis using a 1.9 kB riboprobe derived from rat TH identified mRNA species of 5.5 and 5.3 kB in NIH-3T3 cells transfected with HTH-2. The size of endogenous rat adrenal TH mRNA was approximately 2.0 kB. Western blot analysis using antiserum against bovine TH revealed an immunoreactive band with an apparent molecular weight of 62 kD in these same cells. TH-like immunoreactivity was present in culture in the 3T3 cells containing the HTH-2 gene, but not in control cells. HTH form 2 was characterized using a modification of the assay described by Nagatsu et al. (1964). The V_{max} of the Form 2 cell line ranged from 125 pmol/mg/min. The $K_{m's}$ for L-tyrosine and the pterin cofactor, BH_4, were 101 and 25 µM, respectively. TH activity was inhibited by 3-iodotyrosine, with a K_i of 0.48 µM.

When HTH2-3T3 cells were grown in media supplemented with L-tyrosine (1 mM) and BH_4 (1 mM), the concentration of L-DOPA in the incubation media was 16 µM after 24 hours. L-DOPA production required BH_4 and was blocked by addition of a competitive inhibitor of TH, 3-iodotyrosine.

These results demonstrate that the human TH gene can be transferred to eukaryotic cell lines and that active tyrosine hydroxylase is produced. The efficacy of utilizing these transplantable cells producing micromolar concentrations of L-DOPA in the treatment of dopamine-deficiency states is being investigated.

534.11

KIRSTEN-ras INFECTED PC12 CELLS SURVIVE BETTER THAN NAIVE PC12 CELLS AFTER INTRACEREBRAL TRANSPLANTATION IN ADULT RAT. O.Okuda* and M.W.Brightman. Lab of Neurobiology, NINDS, NIH, Bethesda, MD 20892

The rat pheochromocytoma cell line PC12 can be induced to express neuronal features by Kirsten-ras virus infection (Noda et al., Nature 318:73). The K-ras infected PC12 cells have extensive neurites and contain choline acetyl transferase (ChAT) and tyrosine hydroxylase (TH), indicating both cholinergic and adrenergic features.

K-ras infected PC12 cells were injected into the caudate - putamen of adult Sprague-Dawley rats and the same number of naive PC12 cells were transplanted into the opposite striatum as a control. At two weeks after transplantation, there was usually a big hemorrhagic lesion associated with the naive PC12 cells. In contrast, the K-ras infected PC12 cells appeared as a mass of cells with less inflammatory reaction from the host tissue. By four weeks, grafted naive PC12 cells had disappeared from the host brain in most cases while some K-ras infected PC12 cells consistently remained in all specimens. These surviving K-ras infected PC12 cells were positive for both ChAT and TH and had cellular processes.

K-ras infected PC12 cells survive better than naive PC12 cells after intracerebral transplantation in adult rats and may serve as a continuous source for both cholinergic and adrenergic transmitters without the need of exogenous, nerve growth factor stimulation.

534.12

L-DOPA PRODUCING FIBROBLASTS GRAFTED IN A RAT MODEL OF PARKINSON'S DISEASE. L.J. Fisher, H.A. Jinnah, M.B. Rosenberg, P.J. Langlais, T. Friedmann*, F.H. Gage. Depts of Neuroscience and Pediatrics, University of California San Diego, La Jolla, CA 92093.

We wish to determine the potential therapeutic effect of grafting cells genetically modified to produce catecholamines into the brain of rats with experimental Parkinson's disease (c.f. Neurosci. abst. 11:734, 1988). A fibroblast cell line derived from Fischer rats (Rat1) was infected with a Moloney murine leukemia-based retrovirus vector containing a full length cDNA for rat tyrosine hydroxylase (TH). A clone, Rat1/TH19, that expressed the highest level of TH activity as assessed by a DOPA decarboxylase-coupled assay (3-4 pmol/min/mg protein) was selected for grafting. These Rat1/TH19 fibroblasts exhibited relatively uniform TH immunoreactivity in vitro using a monoclonal antibody to TH. Control uninfected cells did not have detectable TH activity by either biochemical or immunohistochemical assays. When grown in medium containing the natural pterin cofactor for TH, Rat1/TH19 cells produced L-DOPA and released it into the culture medium. Studies are currently under way to examine the long-term survival and behavioral effects of these TH-infected cells when implanted into the striatal parenchyma of Fischer rats with unilateral lesions of the nigrostriatal pathway.

534.13

RAT PC12 CELLS CARRYING THE MOUSE BETA-NGF GENE SURVIVE AND DIFFERENTIATE FOLLOWING IMPLANTATION INTO THE ADULT MOUSE STRIATUM. L.A. Cunningham[1], M.P. Short [2*], U. Vielkind[1*], S. Koh[1], X.O. Breakefield [2], and M.C. Bohn [1]. [1]Dept. Neurobiology and Anatomy, University of Rochester, Rochester, NY 14642 [2]Dept. Neurology, E.K. Shriver Ctr., Mass. General Hospital, Waltham, MA 02254.

Rat PC12 cells infected with a retroviral vector containing cDNA encoding mouse beta-NGF, become amitotic and extend neuritic processes *in vitro* in a manner similar to PC12 cells treated with exogenous NGF. The present studies were undertaken to determine whether these cells continue to express neuronal characteristics following implantation into the striatum of adult mice in which striatal dopaminergic fibers were lesioned with 1-methyl-4-(2'methylphenyl)-1,2,5,6-tetrahydropyridine.

Twenty days post-implantation, many grafted cells survived as assessed by immunoreactivity for tyrosine hydroxylase (TH) and the rat NGF receptor (NGFR). Although most of the genetically modified cells were immunoreactive for TH *in vitro* prior to implantation, at twenty days post-implantation many of the NGFR-immunoreactive cells were not immunoreactive for TH, suggesting a change of neurotransmitter phenotype following implantation. Interestingly, many NGFR-immunoreactive cells displayed a neuronal morphology and extended neuritic processes, especially those at the periphery of the graft site. Thus, PC12 cells genetically modified to synthesize NGF can survive implantation and differentiate within the adult CNS, suggesting their possible usefulness as a replenishable source of donor cells to replace damaged adult CNS neurons.

Supported by NIH grants NS25778, T32MH18260, NS01251, NS24279.

534.14

TRANSPLANTATION AND GENE EXPRESSION IN THE RAT NIGROSTRIATAL DOPAMINE SYSTEM. L.C. Kale*, J.R.Sladek,Jr., T.J. Collier, D.M. Yurek, and G.A. Higgins (SPON: G.J. Thomas). Department of Neurobiology and Anatomy, University of Rochester School of Medicine, Rochester, NY 14642.

Unilateral 6-hydroxydopamine (6-OHDA) lesions of the substantia nigra (SN) selectively destroy dopaminergic neurons and induce rotational behavior in rats. Fetal grafts of SN into the caudate nucleus reverse the behavioral effects of this lesion. Presently, we are using this lesioned-induced behavior model and neural grafting to evaluate changes in gene expression within the nigrostriatal system. *In situ* hybridization has been used to show the presence of tyrosine hydroxylase (TH) mRNA within grafted dopamine (DA) cells, and preliminary data suggest that the presence of a fetal SN graft in the intact striatum may down-regulate TH gene expression in the ipsilateral host SN. In addition, we and others have shown that disappearance of a specific TH hybridization signal over the lesioned SN coincides with elevated levels of preproenkephalin mRNA in the ipsilateral striatum. This model is being used to study how the reintroduction of DA via neural grafting affects the expression of a variety of genes. For example, does the striatum respond to DA denervation by increasing trophic factor gene expression? One such candidate is nerve growth factor receptor mRNA, which is transiently expressed in the striatum during development. Further studies will be aimed at quantitative measures of gene expression in the nigrostriatal system. Support: N.I.H. MSTP T32 GM07356 (L.K.), the Pew Foundation (J.S.), and a Mallinckrodt Scholar Award (G.H).

534.15

EXPRESSION OF TYROSINE HYDROXYLASE IN TRANSFECTED SCHWANN CELLS. R.R. Johnson*, G.C. Owens*, R.P. Bunge, and K.L. O'Malley. Department of Anatomy & Neurobiology, Washington University School of Medicine, St. Louis, MO 63110.

Several studies have shown that fibroblasts can be genetically engineered to express a foreign gene and that such cells can compensate for experimental deficits in brian graft studies. Further work is required to establish that foreign gene expression can be maintained and/or whether cell divisions can be suppressed in long term grafts. Alternative cell types might be more effective in expressing and secreting product as well as be more stable in the environment of the brain parenchyma. For these reasons we are exploring the use of Schwann cells for the insertion and subsequent expression of foreign genes. To determine whether Schwann cells may be able to act as a useful source of DOPA and/or dopamine, we have transfected the gene for tyrosine hydroxylase (TH) into its genome. A TH/retroviral construct was used to create a Psi-2 helper cell line (Wolff et al., Soc. Neurosci. Abstr., 1988). The TH retroviral stocks were used to infect 1.17 Schwann cell cultures (Porter et al., PNAS., 84:7768, 1987) folowed by G418 selection. A number of G418 resistant colonies were obtained and subsequently screened via in situ hybridization for TH gene expression. Results suggested not all G418 resistant cells simultaneously transcribed the gene for TH. Therefore, TH positive cells were further isolated by limiting dilution. RNA purified from one such subclone was subsequently assayed using reverse transcription followed by polymerase chain reaction amplification. Reaction products were Southern blotted and probed with a radiolabeled TH primer. Autoradiographs show comparable levels of hybridization in the 1.17/TH cells and in the PC12 clonal cell line, but not in uninfected 1.17 cells. TH mRNA was effectively translated since either a TH mouse monoclonal/alkaline phosphate sandwich technique or a mouse primary/fluorescent secondary showed immunohistochemically identifiable TH in the 1.17/TH subclone. We are currently evaluating these cells in terms of TH enzymatic activity and for the synthesis and secretion of DOPA. Supported by NMSS RG 1195 A1 and the APDA.

535.1

TROPHIC FACTORS AND THE AGONIST-STIMULATED ACCUMULATION OF INOSITOL PHOSPHATES IN HIPPOCAMPAL SLICES. M.J. Bonner[1], P. Tandon[2], and H.A. Tilson[2]. [1] Curriculum in Toxicology, UNC-Chapel Hill, NC 27599 and [2] LMIN, NIEHS/NIH, Research Triangle Park, NC 27709.

This study helps clarify the actions of different trophic factors (TRF) on the phosphoinositide (PI) system. [^{3}H]-inositol was incorporated into hippocampal slices. Various TRFs were used to stimulate PI metabolism at concentrations between 2 and 2000 ng. At these concentrations NGF, GM1, and mixed GM produced a 5-20% stimulation of IP release. Carbachol produced a 40% stimulation at a concentration of 10-4 M. However, when other TRFs (IGF, EGF, FGF, GM1, or NGF) were added in combination with carbachol, norepinephrine (NE) or 5-hydroxytryptamine (5-HT) it was found that the TRFs produced a general suppression of carbachol-induced IP release, except for NGF which had no effect. NGF and IGF had no effect on NE stimulated IP release, while the other TRFs suppressed IP release. No significant effect on IP release was seen with 5-HT alone. The interaction between different TRFs showed that NGF and FGF together produced a greater release of IP when compared to either of the factors alone. This data indicates that the signal transduction system in the hippocampus is responsive to various TRFs and their interactions with neurotransmitters. (M.B. supported by 5T32 ES07126).

535.2

ACCELERATION OF REINNERVATION and CONVERSION OF SLOW TO FAST TWITCH WITH DENERVATED REGENERATING MUSCLE. ME Clinton, KE Misulis, W-D Dettbarn Neurology & Pharmacology Depts. Vanderbilt Univ. Nashville, TN

Recovery of twitch tension (TwT) after denervation accelerates when regenerating muscle is denervated (DR muscle). We investigated reinnervation of DR muscle.

Rat soleus was studied at 2 & 4 weeks after denervation for (TwT), contraction time, relaxation time and histological fiber typing. Study groups were: proximal or distal sciatic crush, marcaine injected soleus, sciatic crush & marcaine injection, normal controls.

The findings were: soleus assumes physiologic and histologic traits of fast twitch muscle for all groups; marcaine injected muscle reached the same TwT at 4 as at 2 weeks and resumed slow twitch traits by 4 weeks; DR muscle developed higher TwT than denervated muscle at 2 weeks; at 4 weeks there was further increase but denervated muscle TwT matched DR muscle; DR muscle evolved the physiologic & histologic traits of fast muscle.

Rat soleus develops fast twitch characteristics during recovery from denervation or muscle regeneration. Combining denervation and muscle regeneration accelerates reinnervation and increases conversion to fast twitch muscle. These processes may be related and help explain alternative mechanisms of reinnervation.

Supported by NIH-CIDA #NS-011304.

535.3

EFFECTS OF ELECTRIC FIELDS ON REINNERVATION OF THE RAT SCIATIC NERVE. KE Misulis & ME Clinton Neurology & Pharmacology Depts. Vanderbilt Univ. Nashville, TN.

This study examines the influence of electric fields on recovery of physiological and histological characteristics of rat muscle following denervation.

Rats were anesthetized and had the left sciatic nerve transected and reanastomosed using 10-0 ethilon suture. Traxon (R) devices were implanted. These units consisted of a silastic cuff with electrodes attached to either end. The electrodes are connected by flexible wire to the control unit which was implanted subcutaneously. At 2, 3, and 4 weeks later twitch histochemical characteristics were assessed.

Three weeks following initial surgery, twitch tensions of the soleus and EDL were greater in animals with active devices than in animals with inactive (control) devices. There were no significant differences in morphological features of the soleus and EDL between animals with active and inactive devices.

This study indicates that electric fields accelerate the reinnervation of the soleus and EDL following sciatic nerve section and repair. This may prove to be clinically useful for the treatment of nerve injuries.

Supported by NIH-CIDA #NS-01134. Traxon devices supplied by American Biointerface.

535.4

AN INHIBITOR OF PLASMINOGEN ACTIVATION IN SCHWANN CELL CULTURES: FURTHER CHARACTERIZATION AND POSSIBLE INVOLVEMENT IN SCHWANN CELL / NERVE CELL INTERACTIONS. M.B.Clark[1], N.Ratner[3] G.Brouse[1]* and G.M.Dmytrenko[2]. Depts. of Anat.[1] and Neurol.[2], Univ. Md. Sch. of Med., Baltimore 21201 and Dept. of Anat.[3], Univ. Cinn. Sch. of Med., Cinn, OH 45267.

The aims of these experiments were to 1) further characterize an inhibitor of plasminogen activation which we have discovered in conditioned medium from Schwann cell cultures and 2) begin to address the role of plasminogen activator (PA) and its inhibitors (PAIs) in nerve cell (NC) / Schwann cell (SC) interactions. Using SDS-PAGE and reverse zymography we have observed that an inhibitor of PA is present in conditioned medium from NC+SC cultures. The inhibitor was increased in SC cultures from which NCs had been removed suggesting that it was released by SCs and regulated by NCs. It occurs as two bands of Mr = 68 and 72 kD on reverse zymograms after SDS-PAGE. We have now observed a band at Mr = 65 kD identified by specific antibodies to PAI-2 by Western blot. Thus, at least one of the SC PAIs is immunologically similar to PAI-2. Since we had previously shown that SCs also synthesize and release tissue type PA, we have begun to address the question of what function the PA-PAI system may serve in SC / NC interactions. These experiments examined the possible role of PA-PAI in the mitogenic response of SCs to an axonally associated mitogen. NCs cause SCs to proliferate as assayed by 3H-thymidine incorporation followed by autoradiography (Wood and Bunge, Nature 256:662, 1975). We now show that the SC response to an axonal mitogen is decreased significantly in the presence of serine protease inhibitors suggesting that an endogenous serine protease such as PA may be involved in the SC mitogenic response to the axonal mitogen. Since SCs also make PAI, we suggest that this mitogenic response of SCs to axons can be modulated by the endogenous SC PAI. (Supported by PVA-SCRF grant to MBC.)

535.5

RECOVERY OF IMPAIRED SCHWANN CELL - NEURONAL INTERACTION DUE TO NGF DEPRIVATION. B.A. Urschel and C.E. Hulsebosch. Dept. of Anat. and Neurosci. and the Marine Biomed. Inst., Univ. of Tex. Med. Br., Galveston, Tx. 77550.

We have previously shown that neonatal administration of antibodies to NGF alters Schwann cell - neuronal interactions resulting in thinner myelin sheaths on the central processes of primary sensory fibers in the dorsal root (DR) and motor fibers in the ventral root. Since the DR was most effected by the NGF deprivation, this tissue was used to determine if the observed effects were permanent or transient in nature. The relation of myelin thickness to fiber diameter of myelinated axons in T9 DR, was analyzed in animals one week cessation of antibody treatment (ANTI-NGF+1 WEEK). Results indicate that the myelin thickness of small fibers (axon diam. $<2.5\ \mu m$) in the ANTI-NGF+1 WEEK population approached that to the CONTROL population. Therefore, 1) myelination proceeds normally after ANTI-NGF treatment is stopped and 2) the previously observed interruption in Schwann cell - neuronal interactions is a result of NGF deprivation and not an indirect effect due to a general diseased state. (Funded by Bristol-Myers, Florence and Marie Hall Foundation, NS 25450, NS 11255 and NS 01217, NS 07185).

535.6

TARGET REGULATION OF NEUROTRANSMITTER PHENOTYPE OF RAT SYMPATHETIC NEURONS IN VIVO: EVIDENCE FROM PAROTID GLAND TRANSPLANTS. R.J. Schotzinger* and S.C. Landis. Center for Neurosciences, Case Western Reserve Univ., Cleveland, OH 44106

Interactions between neurons and their target organs play an important role in regulating neuronal death and morphology during development. To investigate the influence of target organs on neurotransmitter expression, we have utilized the sympathetic innervation of the rat sweat gland as a model. Early in development, the innervation of the sweat gland expresses only noradrenergic properties e.g., catecholamine fluorescence (CF). As development proceeds, however, these neurons switch from a noradrenergic to a cholinergic phenotype and are characterized by acetylcholinesterase (AChE) staining and choline acetyltransferase (ChAT) activity. In contrast, most other sympathetic neurons remain noradrenergic. In the present study, a transplantation paradigm was employed in which the sweat glands, a sympathetic cholinergic target, were replaced by parotid gland, a sympathetic noradrenergic target. Thus, sympathetic neurons that would normally have innervated sweat gland and developed a cholinergic phenotype innervated a noradrenergic target instead. The fibers which innervated the transplanted parotid continued to exhibit intense CF up to six weeks post-transplantation, the last timepoint studied. Further, neither AChE nor ChAT was observed in the transplants at any timepoint. These results suggest that sweat glands are responsible for the suppression of noradrenergic and induction of cholinergic properties in the neurons which innervate them and provide evidence for a role of target organs in determining neurotransmitter phenotype.

535.7

A NEW METHOD FOR STUDYING TARGET REGULATION OF GENE EXPRESSION IN MOTONEURONS. T.O. Crawford*, P.N. Hoffman, J.W. Griffin (SPON: R.W. Kuncl). Dept. of Neurology, Johns Hopkins Univ. Med. Sch., Baltimore, MD 21205.

Methodologic constraints have limited studies on the role of target muscle or distal axon in regulating motor neuron perikaryal synthesis. An important requirement for meaningful interpretation of any study is the ability to confine analysis to the experimentally manipulated group of neuron cell bodies. We report the development of a double labeling technique that enables semiquantitative analysis of gene expression in a restricted group of neurons defined by their innervation of a single target muscle. We can thus investigate the effect of various manipulations of the target muscle on gene expression in the innervating motoneuron. Prior to an experimental manipulation, a retrogradely transported fluorescent tracer (fluoro-gold) was injected into the target muscle. Following the experiment, animals were perfused with paraformaldehyde and relevant spinal cord sections photographed with fluorescence microscopy to record the innervating motor neuron cell bodies. The same sections were then hybridized in-situ with cDNAs coding for gene products of interest. Cells previously identified by fluorescence labeling were analyzed for grain number and density with a computer-aided video analysis system. Contralateral and different-animal-same-slide controls provided the basis for semiquantitative comparison of gene expression.

535.8

CHICKEN NERVE GROWTH FACTOR RECEPTOR mRNA EXPRESSION IN THE NODOSE GANGLION. F. Hallböök(1) C.S. Ayer-Le Lievre(2), T. Ebendal(3) and H. Persson(1) (1)Dept. Medical. Chemistry, Laboratory of Molecular Neurobiology. (2) Dept. Histology and Neurobiology, Karolinska Institute, Stockholm, (3) Dept. Developmental Biology, Biomedical centre, Uppsala, Sweden.

Neurons in the nodose ganglion has been shown not to be dependent on NGF for survival but rather respond at earlier stages to brain derived neurotrophic factor. However, binding of iodinated NGF to the nodose ganglion has been observed. The expression of nerve growth factor (NGFR) mRNA in chicken epibranchial placode derived nodose ganglion was studied using IN SITU hybridization. Transverse sections of total chick embryos through the nodose ganglion of embryonic day (E) 7, E9, E12 and sections through dissected upper thoracic region and neck of the E15 and E17 embryo, including the nodose ganglion, were hybridized to a 46-mer oligonucleotide complementary to the transmembrane part of chicken NGFR mRNA. The complementary oligonucleotide was used as control probe. Intense hybridization was seen in the early nodose ganglion comparable with the hybridization intensity of the dorsal root ganglia, sympathetic ganglia and the lateral motor column in the spinal cord. Surprisingly, high hybridization levels were also seen in the nodose ganglion at later stages. Labeling with different intensities were mainly observed over large differentiated sensory neurons in the E15 and E17 ganglia. This opens the question of presence and function of NGFR on these placode derived neurons.

535.9

NERVE GROWTH FACTOR RECEPTOR EXPRESSION IN INTRAMUSCULAR NERVES : INDUCTION BY BOTULINUM TOXIN. W-C.Yee. University of Manitoba, Winnipeg, Manitoba R3E 0W3, Canada.

Nerve growth factor receptor (NGFR) expression by Schwann cells following axotomy of peripheral nerves, including motor nerves, led to the postulate that it is triggered by loss of axon-Schwann cell contact. In this study, NGFR expression in intramuscular nerves was examined following pharmacologic denervation by botulinum toxin.

Adult rat soleus and rhomboid muscles were directly injected with botulinum toxin. Thick longitudinal cryostat sections of muscles removed 1 to 28 days later were stained with a combined cholinesterase-NGFR immunocytochemical stain, using a monoclonal antibody to NGFR (192-IgG, courtesy EM Johnson) and a PAP technique.

In control muscles, NGFR staining was generally absent in motor nerves but strongly positive in vascular autonomic and intrafusal sensory nerves. Following botulinum treatment, motor nerves at all time points showed a tubular pattern of staining extending to the motor endplates. When combined with silver staining of axons and compared with S-100 staining of Schwann cells, NGFR staining was localised to Schwann cell surfaces of intact motor nerves.

These findings suggest that factors other than loss of axon-Schwann cell contact may trigger NGFR expression by Schwann cells of motor nerves. Potential factors may be derived from the denervation of muscle or induction of motor nerve sprouting caused by botulinum toxin.

535.10

MEMORY IMPAIRMENT INDUCED BY CHRONIC TREATMENT WITH Fab' FRAGMENT OF ANTISERUM TO NERVE GROWTH FACTOR (ANTI-NGF) IN RATS. T.Nabeshima, S. Ogawa *, H. Nishimura *, T. Kameyama *, R. Takeuchi *,* and K. Hayashi *,*. Dept. Chem. Pharmacol., Meijo Univ., Nagoya 468 and * Dept. Pharmac., Gifu Pharmac. Univ., Gifu 502, Japan.

NGF is a protein of known importance for the development and maintenance of peripheral sympathetic neurons. Recent studies have suggested a possible role for NGF in the central nervous system. It is so far not known, however, whether a deficiency of NGF is responsible for impairments in learning and memory. We report here that continuous intracerebral infusion of a specific anti-NGF over a period of four weeks impairs retention of the Morris's water-maze, passive avoidance and habituation tasks in adult rats. Cannulae atached to Alzet mini-osmotic pumps filled with anti-NGF were implanted into the lateral ventricle. The anti-NGF doses were 6~400 μg per 2 weeks. The pumps were replaced once after 15 days. When the rats were put through the Morris's water-maze task during the anti-NGF infusion period, the distance of swimming and goal latency in the control rats rappidly shortened day by day, compared to that in the anti-NGF-treated rats. The degree of habituation to new environment in the anti-NGF-treated rats was significantly lower than that of the control rats. The step-through latency in the anti-NGF-treated rats was significantly shorter than that of the control rats. The experiments reported here have developed a model in the rat to study physiological changes due to a deficiency of NGF in the adult central nervous system.

535.11

INHIBITION OF PROTEIN SYNTHESIS PREVENTS CELL DEATH IN SENSORY NEURONS DEPRIVED OF NEUROTROPHIC FACTORS. S. A. Scott and A. M. Davies. Dept. of Anatomy, St. George's Hosp. Med. Sch., London, UK SW17 ORE.

During embryonic development many neurons become dependent on a neurotrophic factor, such as NGF, for survival. Recently Martin et al. [J. Cell Biol. 106:829 (1988)] have shown that inhibiting protein synthesis prevents the death of NGF-deprived sympathetic neurons. Thus, NGF may promote neuron survival by suppressing an active cell death program. To determine whether the trophic interactions of other neuronal populations involve similar mechanisms we have examined the effects of inhibiting protein synthesis in sensory neurons dependent on either brain-derived neurotrophic factor (BDNF) or NGF.

Trigeminal mesencephalic (TMN) neurons of E10 chicks, a population of BDNF-dependent proprioceptive neurons, were grown in vitro for 48 hrs in actinomycin D (ACT) or cycloheximide (CHX) to inhibit RNA or protein synthesis, respectively. Both drugs prevented cell death in a dose-dependent manner. ACT maximally rescued TMN neurons at 0.1 ug/ml, the same dose that optimally prevented cell death in NGF-deprived sympathetic neurons (Ibid) and blocked approximately 80% of protein synthesis in E10 DRG neurons. Blocking protein synthesis directly with CHX also rescued BDNF-deprived neurons, but only at doses higher (25-100 ug/ml) than required to rescue sympathetic neurons (Ibid) or maximally inhibit protein synthesis in DRG neurons (1 ug/ml). Neurons from the dorsomedial trigeminal ganglion, a population of NGF-dependent cutaneous neurons, were rescued from cell death by CHX at doses similar to those required by TMN neurons. Thus, both NGF and BDNF appear to promote neuron survival by similar mechanisms. We are further testing the generality of these findings by assessing the effects of protein synthesis inhibition on ciliary neurons deprived of ciliary neuron trophic factor (CNTF).

535.12

NEURONAL EXCITABILITY IS MAINTAINED IN ADULT RAT SENSORY NEURONS CULTURED IN SERUM- AND EXOGENOUS GROWTH FACTOR-FREE MEDIA. Luis G. Aguayo, Forrest F. Weight and Geoffrey White*. Sect. Electrophysiology, LPPS, National Institute on Alcohol Abuse and Alcoholism, Rockville, Md 20852.

It has been suggested that nerve growth factors (e.g. NGF, BDNF) may not be essential for the survival of adult peripheral neurons. In order to investigate neuronal excitability and its regulation by growth factors, we have established a methodology for maintaining and recording from neurons dissociated from adult rat dorsal root ganglia. When cultured in serum-free media, neurons (15-30 µm) survived for over 3 weeks and extended long processes. Whole-cell recordings revealed that the cells had resting membrane potentials negative to -50 mV. Application of depolarizing outward current steps elicited overshooting action potentials (duration < 2.5 ms) in most of the cells examined. The amplitude of the action potential was sensitive to local application of TTX (1µM) but not Cd++ (500 µM). Replacing intracellular K+ with Cs+ greatly prolonged the duration of the action potential. Voltage-clamp recordings demonstrated the presence of inward and outward currents. From a holding potential of -100 mV, depolarizing steps activated a fast inward Na+ current that was sensitive to TTX. With 75 mM external Na+, this current activated at potentials positive to -45 mV and reached a peak at -15 mV. In the presence of internal Cs+ (120 mM) and the absence of external Na+, an inward Ca++ current was observed. Study of isolated outward currents from holding potentials of -50 mV and -100 mV revealed only a sustained K+ current. The amplitude of this current was reduced by 10 mM of TEA and 4-AP.

Our results indicate that adult DRG neurons display electrical excitability when cultured in the absence of exogenous growth factors.

535.13

MONOCLONAL ANTIBODIES TO THE CELL SURFACE AND A SOLUBLE FORM OF HUMAN NERVE GROWTH FACTOR RECEPTOR. M. Clagett-Dame, C. Chung*, M.V. Chao*, J.F. McKelvy and P.S. DiStefano.
Neuroscience Res. Div., Abbott Labs, IL 60064 and Dept. of Cell Biol. and Anatomy, Cornell Univ. Med. Coll., NY 10021.

Monoclonal antibodies (mAb) have been produced to a soluble, truncated form of the human nerve growth factor receptor (NGF-R_t). NGF-R_t was obtained from the conditioned medium (CM) of E9b cells, a transfected rat fibroblast cell line that expresses the human NGF receptor (NGF-R) on the cell surface. (Chao, M. V. et al Science 232:518, 1986). The soluble receptor in E9b CM binds [^{125}I]-NGF, and after chemical crosslinking, a 50,000 Mr protein is immunoprecipitated by a mAb (ME20.4) to the cell surface NGF-R. Using E9b CM as a preparative source, NGF-R_t was partially purified by immunoaffinity chromatography (~100 fold) and used for immunization. Hybridomas were screened by radiometric immunosorbant assay and immunoprecipitation of solubilized cell surface receptor covalently crosslinked to [^{125}I]-NGF. Four positive lines were cloned by limiting dilution and secrete mAb of the IgG_1 subclass. All mAb's bind to the NGF-R and NGF-R_t. Two mAb's immunoblot a single protein corresponding to the NGF-R after resolution of E9b membrane proteins on non-reducing SDS-polyacrylamide gels. All mAb's are specific for human NGF-R and do not cross-react with receptor from other species. Antibody competition studies indicate that three antibodies bind to a similar epitope on the NGF-R_t and one mAb binds to a distinct protein epitope. Antibodies to different epitopes have been used to develop a two site radioimmunosorbant assay to quantitate NGF-R_t in culture medium and human samples.

535.14

DEVELOPMENTAL REGULATION OF TRUNCATED NERVE GROWTH FACTOR RECEPTOR (NGF-Rt) IN HUMAN URINE. P.S. DiStefano, M. Clagett-Dame, D.M. Chelsea*, J.F. McKelvy and R. Loy.
Neuroscience Res. Div., Abbott Labs, Abbott Park, IL 60064, Dept. of Surgery, Univ. of Rochester, Rochester, NY 14642.

Monoclonal antibodies (designated 11F1 and 3G5) recognizing distinct epitopes of human NGF-Rt were employed in a 2-site radioimmunosorbant assay to monitor levels of NGF-Rt in human urine. Urine samples were collected from 70 normal subjects ranging in age from 1 mo to 68 yr. Briefly, 96 well plates were coated with antibody 11F1 and blocked with BSA. Urine samples were added for 1.5 hr, followed by adition of ^{125}I-labeled 3G5 antibody. Levels of NGF-Rt were expressed as fmoles of ^{125}I-3G5 antibody bound/ug creatinine. NGF-Rt levels were highest in 1 mo old urines. By 3 mo NGF-Rt levels decayed to half of those seen at 1 mo. A more gradual decline in NGF-Rt levels was noted between 1-15 yr. Between 15-68 yr values were relatively stable at 5% of 1 mo values. Pregnant women (7-8 mo; n=4) showed significantly elevated levels compared to age-matched normals. Affinity labeling of NGF-Rt with ^{125}I-NGF followed by immunoprecipitation with ME20.4 and gel autoradiography indicated that neonatal urine contained high amounts of truncated receptor (Mr=50kD); decreasingly lower amounts of NGF-Rt were observed on gel autoradiograms with development. NGF-Rt in urines from 1 mo old and 36 yr old subjects showed no differences in affinities for 3G5 or for NGF. These data show that NGF-Rt is developmentally regulated in human urine. These studies establish a groundwork for studying NGF-Rt regulation during the course of various neurodegenerative diseases.

TROPHIC INTERACTIONS III

536.1

NEUROTROPHIC FACTOR-INDEPENDENT SURVIVAL OF AVIAN CRANIAL SENSORY NEURONS. K.S. Vogel* and A.M. Davies. Dept. of Anatomy, St. George's Hosp. Med. Sch., London, UK, SW17 ORE.

Embryonic sensory neurons become dependent on target-derived trophic factors for survival at about the time of target field innervation. We are using the placode-derived neurons of the avian geniculate, vestibular, petrosal, and nodose ganglia to address the question of how trophic interactions are coordinated for neurons that must grow different distances to reach their targets. For the populations of neurons studied, distance between central and peripheral targets decreases in the following order:nodose› petrosal› geniculate› vestibular. To determine whether these neurons differ in their periods of trophic factor-independent survival prior to target innervation, we set up low-density dissociated cultures of ganglia, dissected from E3.5 chicks, in the absence of trophic factors, and monitored survival of individual neurons. We found that sensory neurons differ in their ability to survive in the absence of trophic factors, and that this ability is correlated with distance between central and peripheral targets. Thus, 55-65% of nodose neurons survive 4 days in the absence of trophic factors, whereas less than 5% of vestibular neurons survive after 4 days in the same culture conditions. Intermediate numbers (30-50%) of geniculate and petrosal neurons survive after 4 days in vitro.

To examine the environmental conditions that might influence the acquisition of trophic factor dependency, we exposed E3.5 nodose neurons for 24 hrs to brain-derived neurotrophic factor (BDNF), which supports survival of older nodose neurons, and then removed BDNF from the culture medium. Fewer of the nodose neurons that were thus exposed to BDNF survived, when compared to nodose neurons that were cultured in the absence of BDNF throughout. Thus, exposure to BDNF may influence the acquisition of trophic factor dependency in some nodose neurons.

536.2

TRANSFORMING GROWTH FACTORS α AND ß IN CONTRAST TO EPIDERMAL GROWTH FACTOR STIMULATE SURVIVAL OF SENSORY NEURONS IN VITRO. A.Chalazonitis, J.A.Kessler and R.S.Morrison. Depts. of Neuroscience, Neurology and Neurosurgery, Albert Einstein Coll. Med. and Montefiore Med. Center, Bronx NY 10461.

This study examined the neurotrophic action of several newly identified trophic factors on peripheral neurons. Since sensory ganglion cells have the receptor for epidermal growth factor(EGF), several EGF-receptor binding proteins were examined. Neuronal survival and neuritic outgrowth were analyzed in dissociated cultures of neonatal rat dorsal root (DR), nodose, trigeminal and sympathetic ganglia. EGF (0.1-500 ng/ml) failed to enhance DRG neuronal survival after 11 days in vitro. In contrast, transforming growth factor α(TGFα), significantly enhanced survival with doses as low as 0.1 ng/ml and the effect was maximal at 1-10 ng/ml. Saturating concentrations of EGF did not antagonize the neurotrophic action of TGFα, implying that TGFα doesn't interact with the EGF receptor on DRG cells. Transforming growth factor ß (TGFß), which doesn't bind to the EGF receptor, produced striking changes in DRG non-neuronal cell morphology. TGFß (5 ng/ml) increased survival of DRG neurons at least in part by promoting release of NGF in contrast to TGFα, whose survival effect was not mediated by NGF release. TGFα and EGF had no effect on survival of nodose, trigeminal or sympathetic ganglion neurons. These data indicate that TGFα and TGFß exert specific and distinct neurotrophic effects on subpopulations of DRG neurons.

536.3

EPIDERMAL GROWTH FACTOR AND TRANSFORMING GROWTH FACTOR ALPHA STIMULATE SURVIVAL OF NEURONS FROM MULTIPLE REGIONS OF THE CNS. R.S. Morrison. Albert Einstein Coll. of Med. Bronx, NY 10461

Epidermal growth factor (EGF) and transforming growth factor alpha (TGFα), an EGF-receptor binding protein structurally and functionally related to EGF, have been identified in adult rat and mouse brain. EGF has been shown to promote the survival of rat striatal neurons in culture suggesting that EGF may function in neuronal development. The purpose of this study was to characterize the response of other CNS neuronal populations to EGF and to determine if TGFα is an active neurotrophic agent for CNS neurons. EGF(10 ng/ml) enhanced survival and neurite outgrowth from primary neuronal cultures established from frontal cortex(cx), parietal cx, occipital cx, entorhinal cx and septum of newborn rats. TGFα (0.1-10 ng/ml) enhanced the survival of the same neuronal populations. Both factors stimulated a three fold increase in the average length of neuronal processes. Although both factors stimulated cells from the same brain regions, the response of CNS neurons to these two EGF-R binding proteins was not identical as TGFß (1 ng/ml) antagonized the neurotrophic action of TGFα, but had no effect on the action of EGF. TGFß alone had no effect on CNS neuronal survival. The data suggest that EGF and TGFα may be required for the development and maintenance of CNS neurons. Furthermore, they may either initiate their actions through distinct receptors or be processed differently after binding to a single EGF-R.

536.4

BASIC FGF PROTECTS NGF RECEPTOR IMMUNOREACTIVE NEURONS IN THE BASAL FOREBRAIN AFTER AXOTOMY F. Gómez-Pinilla, J. Phan and C. W Cotman. Department of Psychobiology, University of California, Irvine, CA 92717

Fimbria-fornix transection in rats destroys the main efferents from the medial septum (MS) and vertical limb of the diagonal band (vDB) nuclei to the hippocampus, and causes severe neuronal loss in both the MS and the vDB. It has been shown that chronic treatment with nerve growth factor (NGF) or basic fibroblast growth factor (bFGF) following the transection ameliorate the death of cholinergic neurons in MS-vDB complex. This study investigates the possibility of an interaction between the roles of NGF and bFGF in protecting neurons against death after axotomy. We transected the right fimbria-fornix by a knife cut. A cannula connected to an osmotic mini-pump (Alza 2002, 0.5 µl/h rate) to deliver bFGF (500 ng/ml) or saline, over 14 days, was implanted into the right ventricle. We used an affinity purified monoclonal antibody against NGF receptors (IgG 192, kindly provided by Dr. E Johnson, Jr.) or affinity purified anti GFAP, together with ABC immunohistochemistry. Saline treated controls showed a 40% NGFR neuronal survival in the MS-vDB complex. bFGF treatment ameliorated death of neurons (63% survival in the MS and 82% survival in the vDB) that expressed NGFR immunoreactivity. Astrocytic population increased on the lesion side MS-vDB complex for both the bFGF treated rats and the saline control rats. The rate of astrocytic increase was about the same for both groups, suggesting that the bFGF effect is not mediated by astrocyte-made NGF. Our results suggest that the same neurons that respond to bFGF treatment are susceptible to the action of NGF, and an interaction between the two growth factors is possible.

536.5

NEUROTROPHIC ACTIONS OF ACIDIC AND BASIC FIBROBLAST GROWTH FACTORS ON SPINAL CORD NEURONS. SR Whittemore, PM Sweetnam, HR Sanon*, BB Brass[1], PF Strang[2]. Depts Neurological Surgery, Physiology & Biophysics[1], & Pharmacology[2], Univ Miami Sch Med, Miami, FL 33136.

Under conditions which minimize substrate effects ($7x10^5$ cells/cm^2), effects of aFGF and bFGF on Ei2.5 spinal cord choline acetyltransferase (ChAT), glutamic acid decarboxylase (GAD), and aspartate aminotransferase (AAT) activities were determined. aFGF had no effect on ChAT, GAD, or AAT (0.1-100 ng/ml) in cultures grown with or without FUDR, ± heparin. aFGF did increase protein 58% in mixed neuron-glia cultures. bFGF increased ChAT activity (EC_{50}- 50ng/ml) by 40% in the absence or presence of FUDR, and slightly increased GAD and AAT.

Western blots could not detect aFGF. 17kDa, 30kDa, and 50kDa (≈30, 300, and 600 pg bFGF/µg protein) forms of bFGF were detected, depending on the antibody used. Synthesis of all forms of bFGF was decreased, but not eliminated in cultures grown in FUDR. The nature of the larger protein is under examination. The 17kDa form appeared complexed with ECM proteins. The remaining bFGF was intracellular. Inhibition of proteoglycan synthesis decreased levels of all bFGF forms.

Results suggest bFGF enhances multiple neuronal enzyme activities by a common mechanism of action which may be mediated through bFGF complexed with astrocyte ECM.

536.6

EPIDERMAL GROWTH FACTOR AND BASIC FIBROBLAST GROWTH FACTOR AFFECT OVERLAPPING POPULATIONS OF NEOCORTICAL NEURONS. H.I. Kornblum, H.K. Raymon, R.S. Morrison, K.P. Cavanugh*, R.A. Bradshaw, E.M. Leslie, U.C. Irvine, Irvine, CA. 92717 and Albert Einstein College of Medicine, Bronx, NY. 10467.

Recent studies have indicated that epidermal growth factor (EGF) and basic fibroblast growth factor (bFGF) serve as trophic factors for CNS neurons. In the present study, we demonstrate that neocortical neurons respond to both bFGF and EGF. However, bFGF appears to affect a larger subset of neurons. Dissociated neuronal cultures were prepared from newborn rat brains and plated in DMEM/F12 medium supplemented with 10% fetal calf serum on polylysine-coated plates. One day after plating, cultures were switched to a chemically defined medium and appropriate growth factors were added. Dose response studies indicate a maximal effect on process outgrowth and cell survival with 10 ng/ml EGF and 30 ng/ml bFGF. bFGF had an effect on approximately 15% more neurons than EGF. The combination of EGF and bFGF induced only slightly higher effects on process outgrowth and cell survival, than with either alone. Neuronal survival was maintained in cultures grown in the presence of EGF and then switched to bFGF, while EGF had a lesser effect on cultures grown in the presence of bFGF. EGF and bFGF had differential morphological effects on cortical neurons. bFGF induced a greater number of processes per neuron than did EGF at maximal stimulatory doses. These data indicate that EGF and bFGF support the survival and process outgrowth of an overlapping population of neurons with bFGF affecting a greater percentage of plated neurons. *This work was supported by NIH NS 19319.*

536.7

UNINJURED BASAL FOREBRAIN CHOLINERGIC NEURONS ARE NOT DEPENDENT UPON LIMITING AMOUNTS OF TARGET-DERIVED FACTORS FOR SURVIVAL IN THE ADULT. N.P. Gallety*, C.N. Svendsen*, O. Isacson and M.V. Sofroniew. Department of Anatomy, University of Cambridge, U.K.

Target-derived factors are thought to regulate in a dose dependent and limiting manner, the number of afferent neurons which survive during development. Whether or not such factors continue to regulate the survival of neurons in the adult CNS is not known. To investigate this, NMDA was injected into the hippocampus (Hp) in 6 sites on one side, in young adult rats. After 7-120d, Hp were stained for AChE, Nissl, GFAP and neuron specific enolase, and the septal region for ChAT and NGF receptor. Some NMDA-lesioned rats had complete unilateral transections of the fimbria-fornix (FF) 14d prior to perfusion. Fluorescent tracers were used to determine collateral projections. Between 7-90d after NMDA injections, the Hp exhibited pronounced gliosis, few identifiable neurons and gradually reduced in volume until virtually no cellular elements remained. From 90-120d (n=12), in spite of the absence of cellular elements from the Hp itself, a thin band of white matter containing AChE positive fibers persisted along the entire FF. At all survival times, there was no loss of cholinergic neurons in the medial septum on the NMDA-lesioned side compared with the unlesioned side, in marked contrast to the massive loss (>70%) seen after axotomy in the FF (the sole projection in most cases). These findings indicate that target-derived factors do not regulate the survival of uninjured basal forebrain cholinergic neurons in a dose dependent and limiting manner in young adult rats. Furthemore, proximal axotomy of these neurons may result in cell death by means other than simply depriving them of target-derived trophic support.

536.8

A DEFINED MEDIUM CULTURE SYSTEM FOR EXAMINING TROPHIC EFFECTS ON SEPTAL NEURONS. I. MORPHOLOGIC ANALYSES. G. D. Crawford, R. J. Bostwick,* S. H. Appel. Dept. of Neurology, Baylor College of Medicine, Houston, Texas.

Dissociated septal cultures are a useful model for studying neuronal development. A culture method has been defined which permits good neuronal survival and substantial differentiation while greatly depressing glial growth. The use of antimitotic agents is not required. Greater than 98% of the cells are neuron-like as identified by morphology and neurofilament immunoreactivity. Among the minor cell population are type II-like glial cells identified by morphology and GFAP immunoreactivity. The survival of neuron-like cells is critically dependent on the plating conditions and cell density. The cell culture can be maintained in a state of constant process extension for over one week. The effects of several known trophic factors as well as other substances will be reported. In addition to increasing the number of cholinergic cells displaying ChAT immunoreactivity, NGF also increases the number of well developed Type II glial cells. FGF has a prominent glial mitogenic effect. Heparin profoundly alters the morphology, causing the cell bodies to aggregate and form fascicles, thereby preventing detailed morphometric analysis. This system will allow a systematic in vitro analysis of trophic agent effects on neurons.

536.9

A DEFINED MEDIUM CULTURE SYSTEM FOR EXAMINING TROPHIC EFFECTS ON SEPTAL NEURONS. II. BIOCHEMICAL CHARACTERIZATION OF CHOLINERGIC DEVELOPMENT. J. R. Bostwick,* G. Crawford and S. H. Appel. Dept. of Neurology, Baylor College of Medicine, Houston, Texas 77030

Phosphoethanolamine (PEA) has recently been shown to enhance acetylcholine synthesis in explant cultures from the septal nucleus. We measured the effects of PEA, NGF, basic and acidic FGFs, and heparin on cholinergic development in dissociated septal cells grown under defined conditions. Individually NGF and basic FGF, but not PEA, acidic FGF or heparin, stimulated ChAT activity. Heparin activated acidic FGF to become the most potent enhancer of ChAT activity and potentiated the effect of basic FGF but not that of NGF. PEA did not stimulate ChAT activity when added in addition to any of the peptide growth factors with or without heparin. The effects on ChAT activity were evident by day 2 in culture, were dose-dependent and saturable, and continued to increase for at least 7 days. Effects on ACh synthesis were not evident until the cells had been cultured for at least 7 days. ACh synthesis was stimulated by PEA in a dose-dependent manner which was additive to any stimulation by the peptide growth factors. Heparin potentiated the effects of both basic and acidic FGF but abolished the action of PEA alone or in addition to NGF. The differential effects of these various agents on cholinergic development suggests different modes and specificities of their actions on this cell population.

536.10

COMPARISON OF THREE FACTORS INDUCING CHOLINERGIC PROPERTIES IN CULTURED SYMPATHETIC NEURONS. M. Rao, S. C. Landis, P. H. Patterson, Div. of Biology, Caltech, Pasadena, CA and Center for Neurosciences, CWRU, Cleveland, OH.

Rat superior cervical ganglion (SCG) neurons in culture can express cholinergic properties under the influence of several factors including cholinergic differentiation factor (CDF) from heart cell conditioned medium (HCM), ciliary neuronotrophic factor (CNTF) from rat sciatic nerve and membrane associated neurotransmitter inducing substance (MANS) from rat spinal cord. To determine if these factors are related, we have compared their biological activity in several assays and used antisera generated against CDF in immunoprecipitation studies. As previously reported by Rohrer, Kessler and Fukada respectively, CNTF, MANS and CDF all show cholinergic inducing activity when added to SCG cultures. In addition, both crude and purified preparations of CNTF and MANS promote survival of E8 chick ciliary neurons in culture. In contrast, CDF does not promote ciliary neuron survival. Polyclonal antibodies generated against CDF (Fukada, 1986) immunoprecipitate a 45kD protein from I^{125}-labelled HCM fractions. In similar experiments with labelled CNTF and MANS fractions, no specific protein bands are precipitated. In bioassays, neither the cholinergic inducing activity nor the ciliary neuronotrophic activity of CNTF and MANS can be precipitated by CDF antiserum at concentrations that will immunoprecipitate all cholinergic inducing activity from HCM. Thus, CDF is biologically and immunologically distinct from CNTF and MANS. Supported NIH, McKnight Foundation and the AHA.

536.11

DEVELOPMENTAL REGULATION OF NERVE GROWTH FACTOR RECEPTOR (NGF-R) GENE EXPRESSION IN RAT CNS. Sookyong Koh and Gerald A. Higgins. University of Rochester Medical Center, Rochester, NY 14642.

During CNS development, there is a peak of NGF synthesis in the projection areas of basal forebrain neurons. This developmental change in NGF production is paralleled by an increase in the perikaryal size of NGF-responsive basal forebrain neurons during the third postnatal week, suggesting a possible causative role of endogenous NGF in the mediation of basal forebrain neuronal hypertrophy. In order to determine whether an induction of NGF-R gene expression occurs during this period of hypertrophy, we have used *in situ* hybridization to study the regulation of NGF-R mRNA within individual neurons of rat basal forebrain. ^{35}S-labeled antisense NGF-R RNA probe was generated by *in vitro* transcription of a 1.7 kb cDNA, size-reduced by alkaline hydrolysis, and hybridized under high stringency conditions to pretreated brain sections at postnatal day (P)1, P5, P10, P15, P22, and P30. Increases in NGF-R mRNA content can be observed at P5, is more marked at P10, and reaches almost four times the level of mature neurons by P15. The peak of expression is followed by an abrupt fall in the message level at P22, and the further decrease at P30. Induction of NGF-R mRNA may render the cells more responsive to NGF and augment a cascade of NGF-mediated trophic actions to result in neuronal hypertrophy. In developing cerebellum, the highest levels of message are detectable between P1 and P10 in Purkinfe cells and the external granule layer, suggesting that Purkinje cells and immature granule cells may be sites of NGF-R biosynthesis.

536.12

ENZYME EXPRESSION BY LURCHER MUTANT BERGMANN GLIA IN CHIMERIC MICE DEPENDS ON PROXIMITY TO PURKINJE CELLS. M. Fisher, Department of Anatomy & Cell Biology, Box 439, Univ. Of Virginia School of Medicine, Charlottesville, VA 22908

The role of Purkinje cells (Pc) in regulating aspects of postnatal maturation of Bergmann glia (Bg) in mouse cerebellum has been examined using histochemical and immunocytochemical techniques in Lurcher (Lc) mutant and chimeric mice. Bg maturation is marked by increased expression of glycerol-3-phosphate dehydrogenase (GPDH) and by concentration of histochemically demonstrable β-glucuronidase (β-glu) into a dense supranuclear cap. Both processes appear to reverse themselves in Lc/+ mice following Pc death. In contrast, these enzymes' expression in other cerebellar cell types does not appear to be altered in mutant mice. The present study demonstrates that in chimeras that are Lc/+, gus^b/gus^b (i.e., high level β-glu) <--> +/+, gus^h/gus^h (i.e., low level β-glu), both normal and Lc/+ Bg are GPDH-immunopositive and show normal β-glu histochemistry when they are close to surviving Pcs. In contrast, all Bg in Pc-free gaps are GPDH-immuno negative and fail to show mature β-glu histochemistry. These observations indicate that altered expression of GPDH and β-glu seen in Lc/+ mice is a secondary effect of Pc death and does not reflect intrinsic defects in the Lc/+ glial cells. Supported by NS25350.

536.13

REGULATION OF CATECHOLAMINERGIC PHENOTYPE OF PC12 CELLS BY CELL CONTACT AND NGF. H. Badoyannis*, S.C. Sharma, & E.L. Sabban (SPON: S. Fraley) NY Med Coll. Valhalla, NY 10595

Tyrosine hydroxylase (TH), the first and rate-limiting enzyme for catecholamine biosynthesis, is subject to regulation by cell contact and NGF. It has been shown that TH mRNA levels were induced in PC12 cells grown at high densities whereas NGF treatment decreased TH mRNA levels. Regulation of TH alone, however, does not specify the particular catecholaminergic phenotype. Therefore, we examined dopamine β hydroxylase (DBH) mRNA levels in PC12 cells grown at various cell densities, and/or for various times with NGF (50ng/ml). Quantitative Northern or slot blot analyses revealed that at 10 ∿ 20-fold higher densities there was up to an 18-fold increase in TH mRNA levels, but DBH mRNA levels decreased slightly (∿30%). There was a 5-fold increase in cellular dopamine content but virtually no change in norepinephrine levels as measured by HPLC-EC. NGF treatment for up to 8 days led to a decrease in TH mRNA levels (∿60%) while DBH mRNA levels rapidly decreased by as much as 85%. NGF treated PC12 cells (4 days) replated to high density still demonstrated an induction of TH mRNA, however, DBH mRNA levels decreased to undetectable levels. Thus, increased cell contact differentially regulated TH and DBH mRNA levels suggesting that cell contact promotes a more dopaminergic phenotype whereas NGF treatment of PC12 cells decreases both TH and DBH mRNA levels.

536.14

VISUAL RESPONSES OF AXOTOMIZED GANGLION CELLS ARE PRESERVED BY EMBRYONAL NERVOUS TISSUE TRANSPLANTS. L. Domenici*, A. Gravina*, N. Berardi*, L. Galli* and L. Maffei.* (SPON: C. Lance-Jones) Istituto di Neurofisiologia del CNR, 56100 Pisa, Italy.

We evaluated the ability of embryonal nervous tissue transplants to preserve the functional integrity of axotomized retinal ganglion cells by recording the visual retinal responses in rats after unilateral intraorbital section of the optic nerve with and without transplant of embryonal nervous tissue directly on its stump. Visual retinal responses were evaluated by pattern electroretinogram (ERG). We found that the pattern ERG recorded in the eye with the optic nerve section and embryonal nervous tissue transplant was almost indistinguishable from the pattern ERG recorded in the normal eye even four months after surgery. By this time the pattern ERG has almost completely disappeared in the rats with optic nerve section and no transplant. Correspondingly, four months after surgery a greater number of ganglion cells was found in the cresyl violet stained retinae from the eyes with optic nerve section and embryonal nervous tissue transplant than in the controls with only optic nerve section.

536.15

THE ROLE OF GANGLIOSIDE GM1 IN MEDIATING NEUROTROPHIC INTERACTION. D.F. Chen*, K.C. Leskawa* & F.J. Roisen (SPON: B. Rigor). Dept. of Anat. Sci. & Neurobiol., Univ. of Louisville, Sch. of Med., Louisville, KY 40292.

Exogenous gangliosides potentiate the action of several neurotrophic factors including Nerve Growth Factor (NGF) and factors in glial conditioned medium (GCM). Previously, we have shown that ganglioside GM1 potentiated NGF-mediated and GCM-mediated neuritogenesis of chick embryonic sensory ganglia (DRG) during development. No GM1 potentiation was observed during the peak of NGF-mediated development, embryonic day (ED) 8 to 10; on other days, treatment with GM1 and NGF enhanced growth. In contrast, maximal potentiation of GCM by GM1 occurred on ED 9-10. To determine whether the differences in GM1 sensitivity were related to levels of endogenous GM1, gangliosides were measured in DRG (ED 7-13) by incorporation of [^{3}H]-galactose and GM1 binding of B-cholera toxin-horseradish peroxidase. The synthesis of total gangliosides declined during development. The concentration of GM1 was specifically high at ED 7 and dramatically decreased to its lowest levels during ED 8-10. After ED 11, GM1 content increased markedly. Therefore, at days when endogenous GM1 content was lowest, exogenous GM1 did not potentiate NGF action, but did maximally potentiate the action of GCM. The results suggest that the mechanisms by which GM1 potentiates neurotrophic factors differ with each factor. Supported by NIH NS24524, DE07734 and NS21057.

536.16

A SEROTONERGIC NEUROTROPHIC FACTOR IS INDUCED IN STRIATUM BY LESIONING DOPAMINE NEURONS IN SUBSTANTIA NIGRA OF ADULT RAT F.C.Zhou and J. M. Murphy. Dept. of Anatomy and Psychiatry, Indiana Univ. Sch. of Med., Indianapolis, IN 46223.

We have previously extracted a 5-HT neurotrophic supernatant from the 5,7-DHT lesioned hippocampus (Zhou et al, J. Neurosci. Res, 17:235, 1987). Current study shows that a new 5-HT neurotrophic signal was monitored in a DA-5-HT-striatal model.

A 6-hydroxydopamine (6-OHDA , 50ug/10ul ascorbic saline, two ipsilateral injections) lesion in the nigra specifically removed the dopamine (DA) neurons in nigra and majority of DA-terminal fibers in striatum. In response to the DA-denervation, with immunocytochemical staining, we found that the 5-HT fiber grows vigorously in the previously sparsely innervated striatum. The 5-HT fibers, normally innervating the striatum sparsely and the pallidus densely with sharp delineation (in control side), became dense across both areas with no appreciable delineation (in lesion side). The increase of 5-HT fibers was more prominent in posterior than in anterior striatum.

HPLC measurement supported this view. Significant increase in 5-HT and 5-HIAA level was evident in the posterior striatum when the decrease of DA level exceed 90% in nigra and striatum. In addition, we found that when the decrease of DA level in substantia nigra and striatum was less than 90%, the 5-HT and 5-HIAA levels were not significantly changed in the striatum or pallidus. The induction of 5-HT sprouting requires a >90% decrease of DA level.

Furthermore, the 6-OHDA induced 5-HT neurotrophic signal was also monitored by brain tissue transplant technique. Dissociated fetal raphe rich in 5-HT neurons survived in a significantly higher number in the striatum of lesion side than in contralateral side. These evidences indicates a trophic signal was induced when more than 90% of DA content were depleted. This trophic signal induces sprouting of adult 5-HT fibers and increases the survival of fetal 5-HT neurons. Sponsored by NIH Grant 23027.

536.17

c-FOS GENE EXPRESSION FOLLOWING CORTICAL INJURY AND INTRACEREBRAL INJECTIONS OF NGF AND EGF. F.R. Sharp, M.F. Gonzalez, and S.M. Sagar, Depts of Neurology and Physiology, UCSF and VA Med Ctr, SF, CA 94121.

Small lesions of rat motor cortex induces Fos-like immunoreactivity and c-fos mRNA expression, assessed using in situ hybridization, in neurons throughout the ipsilateral neocortex. To explore the possible involvement of growth factors in this injury induction of the c-fos proto-oncogene, NGF (3ug) was injected into rat motor cortex. This induced Fos, the protein product of the c-fos gene, in neurons throughout neocortex 24 hours after the injection. Saline control injections had little effect. EGF (3ug) was also injected into rat motor cortex and hippocampus. This growth factor induced Fos in presumptive oligodendroglia in corpus callosum, in endothelial cells in cortex and hippocampus, and perhaps in astroglia around the local site of injection.

These data suggest that trophic factors induce Fos in an anatomic pattern that depends on the nature of the trophic factor and receptors on the target cells. The injury induction of Fos throughout neocortex is consistent with the local release of NGF and EGF at the cortical injury site. Though the cellular function of Fos is still unknown, these results show that trophic factors can produce diffuse, prolonged Fos-mediated metabolic changes in neurons throughout neocortex.

536.18

PROGESTERONE PROMOTES SURVIVAL OF AXOTOMIZED MOTONEURONS. Wan-hua Amy Yu. Dept. of Cell Biol. Anat. Sci., City Univ. of New York Med. Sch., New York, NY 10031.

The magnitude of axotomy-induced neuronal loss in adult female rats could be attenuated by the administration of testosterone or progesterone (Yu, W.H.A., J. Neurosci.; Brain Res., in press). The present study investigates whether progesterone (P) acts as a precursor for testosterone (T) or P is effective by itself in reducing neuronal loss. Three-week-old male and female rats were subjected to unilateral transection of the hypoglossal and facial nerves, and injected s.c. with 0.5 or 2.0 mg P in 0.1 ml sesame oil twice weekly for the first 4 postaxotomy wks and once weekly for 6 additional wks. Controls received oil vehicle injection. Neuronal numbers in the hypoglossal and facial motor nuclei were counted 10-11 wks after axotomy in serial paraffin sections stained with cresyl violet. Results indicate that P treatment with either dose significantly reduced neuronal loss in the hypoglossal nucleus in females, but the same treatment did not alter the magnitude of neuronal loss in males. Since the administration of T to prepubertal male rats adversely caused an increase in neuronal loss similar to the condidtion seen after orchidectomy (unpublished data), the presence of response to P treatment in females but not in males suggests that P promotes neuronal survival not by the enzymatic conversion to T. However, a gender difference in the amount and/or the time course of the acquisition of the conversion enzyme may exist.

536.19

LOCALIZATION OF NGF RECEPTOR IN HUMAN BASAL FOREBRAIN USING TWO NEW MONOCLONAL ANTIBODIES: COMPARISON OF NORMAL AND ALZHEIMER BRAINS. R. Loy, D. Heyer*, M. Clagett-Dame* and P.S. DiStefano. Div. Neurosurgery, Univ. Rochester Sch. Med., Rochester, NY 14642 and Abbott Labs, Abbott Park, IL 60064.

Cholinergic neurons of the basal forebrain respond trophically to nerve growth factor (NGF), suggesting that NGF may play a role in the cognitive deficits associated with Alzheimer's disease (AD). In aged rats, brain NGF and NGFmRNA are both decreased, as is NGF receptor (NGFR) immunoreactivity and [125I]-NGF binding. In human tissue, however, no decline in mRNA for either NGF or NGFR has been found. To evaluate potential changes in NGFR immunoreactivity associated with AD, we have used a monoclonal antibody (mab) to the complete receptor, Me20.4, and two new mabs, 3G5 and 11F1, directed against the truncated form of human NGFR. Both Me20.4 and 11F1 recognize the same epitope, while 3G5 recognizes a distinct epitope on the extracellular component of NGFR. Staining with all three mabs is equivalent in the human basal forebrain, and corresponds closely to the distribution of cholinergic magnocellular neurons, extending caudally from the medial septal nucleus, through the nucleus of the diagonal band of Broca and the nucleus basalis of Meynert (Ch4). In addition, all three mabs reveal a shrunken, vacuolated appearance in Ch4 cells in the AD brain. Morphometric analysis of sections stained with Me20.4 shows a 30% decrease in mean cell area from 1691 ± 80 to 1188 ± 95 ($p < .001$) in region Ch4am.

TROPHIC INTERACTIONS IV

537.1

STUDIES OF PROTEIN KINASE C (PKC) EFFECTORS ON NERVE GROWTH FACTOR (NGF) STIMULATED NEURITE OUTGROWTH IN PC12 CELLS. L. K. Taylor* and K. E. Neet. (SPON: I. R. Kaiserman-Abramof) Dept. Biochem., Case Western Reserve University. Cleveland, OH 44106.

We have previously reported that down regulation of PKC in PC12 cells by phorbol 12-myristate 13-acetate (PMA) treatment does not effect the subsequent outgrowth of neurites when bioassayed with NGF in defined medium [Reinhold & Neet, J. Biol. Chem. 264, 3538 (1989)]. We now extend these studies to other conditions. Neurite outgrowth in defined medium is severely depressed when down regulation with PMA is followed by sphingosine treatment, indicating that sphingosine is probably acting to inhibit a kinase other than PKC. Inhibition of neurite outgrowth by sphingosine, [Hall, et al, J. Biol. Chem. 263, 4460 (1988)] is more pronounced in defined than in serum containing medium, with an IC50 that is about 5-10 fold lower. Sphingosine may either bind to serum proteins or interact with signals from growth factors in the serum. Finally, the macrocyclic lactone bryostatin, an antineoplastic agent that has been shown to affect nuclear protein kinases similar to PKC [Fields, et al., J. Biol. Chem. 263, 8253 (1988)], causes partial inhibition of neurite outgrowth when utilized in a down regulation protocol. Neither PMA nor bryostatin at low concentrations cause significant enhancement of NGF-stimulated neuritogenesis. We conclude that phorbol-sensitive PKCs are not required for neurite generation in PC12 cells, that sphingosine probably acts on other kinases such as calmodulin kinase-III, and that another bryostatin-sensitive PKC may play a role in response to NGF. (Supported by NIH grant NS24380)

537.2

VIP ACTIVATES PROTEIN KINASE C IN NUCLEI ISOLATED FROM RAT HIPPOCAMPUS. C.L. Weill. Dept. of Neurology and Anatomy, Louisiana State University Med. Ctr., New Orleans, LA 70112.

Vasoactive intestinal peptide (VIP) is colocalized, and presumably released with acetylcholine cholinergic neurons. I report here that VIP activates a kinase activity in nuclei isolated from rat hippocampus that has the properties of protein kinase C. Using an *in vitro* assay that monitors the incorporation of ^{32}P into endogenous nuclear substrates in the absence and presence of the protein kinase C inhibitor 1-(5-isoquinolynylsulfonyl)-2-methylpiperazine, H-7, VIP was found to maximally effect a 5 to 6-fold increase in phosphate incorporation relative to background. The response was dose and time dependent, with a statistically significant maxima at 4 min and VIP concentration of 10^{-10} M. The response appeared mediated by protein kinase C as inhibition of the VIP response was observed in the presence of sphingosine and staurosporine. Activation was also observed with the active phorbol esters, phorbol 12-myristate 13-acetate (PMA) and phorbol-12, 13-dibutyrate (PDBu), and the diacyglycerol analog 1-oleoyl-2-acetylglycerol (OAG), while activation was not observed with the inactive phorbol ester, 4α-PMA; sphingosine and staurosporine also inhibited activation by PMA. Nuclear kinase activation was not observed with cyclic nucleotides, and corticosterone, however both NGF and SRIF effected kinase activition but not to the same level observed with VIP. The response to VIP was regionally specific; no stimulation of phosphorylation was observed with isolated nuclei from cortex. These data indicate that VIP activates a pool of protein kinase C in isolated nuclei from adult rat hippocampus.

537.3

OLFACTORY NERVE INGROWTH INDUCES TYROSINE HYDROXYLASE EXPRESSION IN RAT FOREBRAIN NEURONS. K. M. Guthrie, C. M. Gall and M. Leon. Depts. of Psychobiology, and Anatomy & Neurobiology, University of California, Irvine CA 92717

Functional olfactory nerve input is required for the normal expression of tyrosine hydroxylase (TH) by dopaminergic neurons in the glomerular region of the rodent main olfactory bulb. To determine whether olfactory nerve input exerts a similar influence on cells in other brain regions, we allowed the olfactory nerve to innervate the rat forebrain. To accomplish this, we performed unilateral bulbectomies in rat pups on postnatal day 5-7 and examined the brains 2-6 months later, after the regenerated olfactory nerve fibers had penetrated the forebrain. Tissue was stained for TH-, dopamine B-hydroxylase (DBH)-, and olfactory marker protein (OMP)-immunoreactivity. In addition, *in situ* hybridization autoradiography using a ^{35}S-labeled ribroprobe was used to examine the forebrain for TH mRNA.

We observed expression of TH-immunoreactivity and mRNA in neurons located in areas of the forebrain which received ingrowing olfactory nerve fibers, particularly along the rostral extension of the subependymal layer. Many of these neurons resembled the periglomerular cells of the olfactory bulb. No cell staining for DBH-immunoreactivity was observed in these areas, suggesting the possible dopaminergic phenotype of these neurons. Since it takes several weeks for the regenerating nerve fibers to reach the forebrain, this is the first demonstration of induction of novel expression in the mature brain.

537.4

FIRING PATTERN ABNORMALITIES IN CULTURED RAT NEOCORTEX NEURONS AFTER CHRONIC SUPPRESSION OF GABAergic SYNAPTIC ACTIVITY IN VITRO. M.A. Corner and G.J.A. Ramakers, Neth. Inst. for Brain Research, 1105 AZ Amsterdam,

Whereas chronic blockade of action potential generation in developing neocortex cell cultures produces severe functional disturbances (Ramakers and Corner, Eur. J. Neurosci. suppl., 1988), intensification of neuronal activity, using picrotoxin (PTX) to release the network from GABAergic inhibitory regulation, was found to have little effect (Ramakers and Corner, Soc. Neurosci. vol. 13, 1987). We have reinvestigated this question using interval histograms derived from spontaneous spike trains of chronic PTX-treated (1 µM) and control neurons, cultured for three weeks in a chemically defined medium and recorded the day following transfer to PTX-free or fresh control medium. Modal intervals for units in the untreated group fall into three approximately equal classes, with only a few intervening values: i) bursty firing, with modes of less than 10 msec; ii) phasic firing, with modes of 15-25 msec, and iii) tonic firing, with modes greater than 30 msec. In the PTX-treated group, the majority of neurons were "bursty" and only 15% were "phasic" ($p<.02$). In addition, the incidence of bimodal distributions - only 20% in the controls - was more than doubled in the experimental group ($p<.01$). Virtually all of these secondary peaks in the interval histogram were in the "tonic" range, so that chronic PTX treatment had resulted in an overall shift towards very high frequency discharges, but separated by relatively long silent periods in comparison with normal firing patterns. Such a change could have resiulted from a general increase in membrane polarization levels, whereby spikes become more difficult to elicit but, once triggered, tend to occur repetitively in rapid succession. Up-regulation of GABA receptors in the course of the prolonged period of blocked synaptic inhibition could thus account for the observed pathophysiology.

537.5

EXPRESSION OF SODIUM CHANNELS ON HYPOMYELINATED CENTRAL AXONS.

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A deletion at the *shiverer* locus (chr. 18) specifically eliminates myelin basic protein gene expression in murine oligodendrocytes, resulting in loose periaxonal wrappings and absence of compact myelin formation throughout the central nervous system. One unexpected neurological phenotype of the MBP$^-$ hypomyelinated brain is the preservation of motor strength, indicating intact impulse conduction in large-caliber corticospinal axons. To identify the underlying excitability mechanisms, we prepared autoradiograms of frozen 20um brain sections following specific external membrane binding with the sodium channel alpha-subunit ligand ^{3}H-saxitoxin (5nM ^{3}H-STX incubated at 4C in 20mM Tris-HCl buffer, pH 7.4, containing 140mM N-methyl-glucamine, 5.4 mM KCl, 2.8 mM $CaCl_2$ and 1.3 mM $MgSO_4$). Densitometric comparisons of toxin binding patterns revealed striking increases over hypo-myelinated *shi/shi* fiber tracts relative to control +/+ pathways. Binding was particularly elevated in transcallosal cortical association fibers, optic nerves, fimbriae, and corticofugal tracts. Scatchard analysis performed on homogenized *shi/shi* and +/+ optic nerves revealed specific binding to a single site, and a 4-fold increase in the number of *shi/shi* STX sites, with no alteration in toxin affinity. Since few sodium channels normally exist in myelinated axon internodes, these data show that central neurons possess the developmental potential to modify axonal excitability in response to altered axo-glial contact interactions by increasing sodium channel expression.

Supported by NIH 11535, March of Dimes, and Pew Scholars Award (JLN).

537.6

ACTIVITY-DEPENDENT REGULATION OF MUSCLE N-CADHERIN C.G. Hahn* and J. Covault. Department of Physiology and Neurobiology, Univ. of Connecticut, Storrs, CT 06269.

Denervation of muscle leads to the production of factors which promote its reinnervation. Nerve activity suppresses the expression of these factors. The adhesion molecule NCAM is thought to be such a factor, its expression in muscle fibers is down regulated by nerve-evoked activity. We have found that expression of the adhesion molecule N-cadherin is similarly regulated.

N-cadherin is abundant on the surface of embryonic chick myotubes but is markedly reduced within 3-4 days of their innervation. In adult chickens, N-cadherin was undetectable on innervated fibers but was induced by surgical denervation. The effects of denervation could be mimicked by TTX blockade of nerve conduction indicating that N-cadherin expression is normally inhibited by nerve-evoked electrical activity.

Activity-dependent regulation of N-cadherin and NCAM can be reproduced, at least in part, in myotube cultures. TTX blockade of spontaneous activity for 2 days caused a 20-25% increase in N-cadherin and a 30-40% increase in NCAM. The effect of TTX was reversed by the Ca channel agonist ryanodyne suggesting increased intracellular Ca may be involved in the activity-dependent down regulation of N-cadherin and NCAM. Further studies will be required to test the validity of this culture system as a model for the normal regulation of these molecules. (Supported by the Sloan Foundation and NIH)

537.7

MUSCLE PARALYSIS BY BOTULINUM TOXIN CAN PROVOKE SIGNS OF AXOTOMY IN MOTONEURONS. M.J. Pinter, S. Vanden Noven, D. Muccio* and N. Wallace. (SPON: I. Zimmerman). Dept. of Anatomy, Medical College of Pennsylvania, Philadelphia, PA 19129.

We examined the electrical properties of cat spinal motoneurons (MNs) supplying muscles paralyzed with type A botulinum toxin (BTX). A dose of about 25 ng. of BTX (in 1.0-1.5 ml saline) was injected into the medial gastrocnemius (MG) muscle. After 2-3 weeks, the animals were prepared for intracellular recording from MG MNs. Animals were divided into two groups based on the amount of muscle force evoked by nerve stimulation. Twitch tensions of one group (low force) were < 5.0 gms (mean, ≈ 4 gms); the other group (high force) had twitches > 20 gms (mean, ≈ 43 gms). Electrical properties of MG MNs from the low force group displayed axotomy-like alterations (eg., decreased mean rheobase current) while the properties of MNs from the high force group appeared normal. Axonal conduction velocities from both groups were normal. Connectivity was assessed by intracellular stimulation of MG MNs and averaging from multiple pairs of EMG electrodes inserted in the treated MG muscle. EMG signals were detected in 35/102 MNs; 25 of these were from the high force group while 10 were from the low force group. The electrical properties of connected MNs were normal while properties of unconnected MNs appeared axotomy-like. One interpretation of these results is that prevention of transmitter release or loss of functional contact with muscle (or both) can provoke signs of axotomy in MNs in the absence of mechanical damage to the axon. Sponsored by NIH grants NS24000 and NS24707.

537.8

AN ELECTROPHYSIOLOGICAL STUDY OF SLOW FIBRES IN DENERVATED CRURALIS MUSCLE IN VIVO AND IN ORGAN CULTURE. L. Szczupak,*L. Nicola Siri,* A. Mezio* and O.D. Uchitel* SPON: D. Weisblat). Inst. de Biologia Celular, Facultad de Medicina, U.B.A., Paraguay 2155, 2° piso, 1121 Buenos Aires, Argentina.

Both slow and fast fibres have been characterized in the cruralis muscle of the toad Bufo arenarum; normally, the slow fibres, which in this muscle are abundant and easily penetrated by microelectrodes, do not exhibit action potentials, but after denervation of the cruralis, they developed strong action potentials in response to depolarizing currents.

Action potentials occurred in cruralis slow fibres 25 d after the sciatic nerve was transected at hip level. The latency was reduced to 20 d when the nerve was cut where it entered the muscle, and to 18 d by injecting α-bungarotoxin into the muscle at denervation. The development of action potentials also showed a seasonal dependence, occurring in summer and not in winter. Cruralis muscles survived in organ culture for up to 30 days; slow fibres of cultured muscles developed action potentials after 24 d. Slow fibres of denervated muscles changed little in V_{mem} or R_i, but showed a significant decrease in τ, reflecting a decreased capacitance of the cells.

The effect of α-bungarotoxin on the latency of the response suggested that Ach may play a neurotrophic role. But slow fibres of cruralis muscles cultured in the presence of an Ach agonist (1-5 µM carbachol) developed regenerative responses as usual. Our results suggest that synaptic transmission may be important in regulating the excitability of cruralis slow fibres, but that Ach alone does not seem to be the critical factor.

537.9

CHARACTERIZATION OF GIP-70, A GUANETHIDINE-INDUCED, NGF-REGULATABLE PROTEIN EXPRESSED IN SYMPATHETIC GANGLIA. P.T. Manning, N.R. Siegel*, C.E. Smith*, S. Aykent* and R.L. Leimgruber*. Monsanto Co., St. Louis, MO 63198.

Guanethidine is an adrenergic neuron blocking agent. Chronic administration of guanethidine to rats, hamsters and monkeys induces an autoimmune, cell-mediated destruction of sympathetic neurons. Neuronal destruction can be completely prevented by concurrent treatment with nerve growth factor (NGF). We have previously identified a protein, guanethidine-induced protein-70 (or GIP-70), by 2-dimensional gel electrophoresis which is induced in sympathetic ganglia following guanethidine treatment. This protein is not found if rats are treated with guanethidine and NGF concurrently. GIP-70 has been hypothesized to be a candidate antigen involved in this immune response. To further characterize GIP-70, the protein was isolated in pure form from homogenates of sympathetic ganglia from 550 guanethidine treated rats using preparative 2-D gel electrophoresis. The spots corresponding to GIP-70 were excised, electroeluted from the gel, and digested with trypsin. Four tryptic fragments,separated by HPLC, were sequenced using Edman degradation gas phase sequence analysis. All four peptides exhibited significant homology to the deduced sequence of the α,β-interferon-induced mouse Mx protein. In addition, GIP-70 and Mx have similiar molecular weights and isoelectric points. GIP-70 thus appears to be the rat homolog of mouse Mx. Experiments are in progress to determine whether rat Mx protein is involved in the induction of the guanethidine-induced immune response.

537.10

ALTERED SYNAPSE FORMATION AND REPRESSION IN THE STRIATUM OF ANIMALS PERINATALLY EXPOSED TO MORPHINE. A.Gorio, B.Tenconi*&, R.La Croix*&, A.M.Di Giulio& and P.Mantegazza*&.Inst. of Pharmacol. Sciences, School of Pharmacy, via Balzaretti 9 and Istituto Scientifico H.S.Raffaele, via Olgettina 60; &Dept. of Medical Pharmacology, School of Medicine, via Vanvitelli 32, Milano.

Rats exposed to morphine during the entire fetal life and postnatally up to the day of sacrifice were utilized in the study. The main affected area is the corpus striatum. Noradrenaline content in this area is higher at postnatal day 12, but later on its levels normalize. Serotonin is also affected by a moderate transient neonatal reduced innervation of the striatum. The dopaminergic innervation is significantly reduced up to day 30 of life. The developmental pattern of striatal met-enkephalin innervation is markedly affected as well, being the peptide levels much higher than controls up to day 60 of life, thus indicating increased synaptogenesis. The pattern of met-enkephalin synaptic repression is similar in normal and morphine-exposed animals. Substance P shows a transient hyperinnervation of the striatum, limited to the first decade of life.

537.11

A MONOCLONAL ANTIBODY FOR PROTEASE NEXIN 1 (PN-1) IS STRONGLY IMMUNOREACTIVE IN ENDOTHELIAL CELLS AND SMOOTH MUSCLE CELLS OF THE BLOOD VESSELS OF HUMAN CEREBRUM. M. Suzuki*, B.H. Choi, S.L. Wagner* and D.D. Cunningham* (Spon: S. van den Noort) University of California at Irvine, Irvine, CA 92717.

Protease nexin 1 (PN-1), also called glial derived nexin (GDN), is a 43 kd protein protease inhibitor that is secreted by a variety of cultured cells including fibroblasts, smooth muscle cells and astrocytes. Although PN-1 was first purified from conditioned medium of cultured human fibroblasts the presence of relatively large amounts of PN-1 in both human and rat brain has also been demonstrated. A panel of monoclonal antibodies to PN-1 was produced by fusing P3X-myeloma cells with splenocytes from a Balb/c mouse previously immunized with highly purified human PN-1. Supernatants of one of the resulting hybridomas (C1B5), which are found to be specific for PN-1 by both ELISA and western blotting techniques were used for immunoperoxidase staining of paraffin sections of the adult human cerebrum fixed in 2% paraformaldehyde. Highly specific immunoperoxidase reaction of PN-1 is noted in endothelial cells and smooth muscle cells of the blood vessels within the parenchyma as well as leptomeninges of the cerebral cortex. Immunoperoxidase staining using a polyclonal antibody for PN-1 produced almost identical results. It is believed that neurotrophic activity of PN-1 is dependent on its ability to inhibit thrombin, and accumulating evidence suggests that their interplay might be important in the regulation of neurite outgrowth in the brain. Demonstration of PN-1 in endothelial cells and smooth muscle cells of the blood vessels of the brain thus provides an important clue as to the cellular localization as well as to the role of these molecules in development, function and regeneration of the central nervous system. (Supported in part by NIH grants ES-02928, GM-31609 and CA-09054)

537.12

PRODUCTION OF RECOMBINANT EXTRACELLULAR DOMAIN OF HUMAN NERVE GROWTH FACTOR RECEPTOR IN BACULOVIRUS EXPRESSION SYSTEM. P. Vissavajjhala and A.H. Ross. Worcester Foundation for Experimental Biology, 222 Maple Avenue, Shrewsbury, MA 01545

The recombinant extracellular domain (RED) of human nerve growth factor receptor (NGF-R) was expressed in Sf9 (Spodoptera frugiperda) insect cells using a baculovirus expression system. A transfer vector was constructed inserting the cDNA coding for extracellular domain of the NGF-R into pVL941. Recombinant virus was produced by cotransfecting the DNA of Autographa californica multiple nuclear polyhedrosis virus and the transfer vector into Sf9 cells. Recombinant viral plaques were screened for the presence of NGF-R gene and for RED expression. RED-positive virus was plaque purified and used for infection of large scale suspension cultures. The RED was secreted into the cell medium from 48hr to 96hr postinfection and was found to bind ^{125}I-NGF.

RED was purified by ammonium sulfate precipitation, immunoaffinity chromatography and ion exchange chromatography. Approximately, 4-5mg of the RED was produced per liter of suspension culture. The RED has a higher apparent molecular weight by gel exclusion chromatography than predicted suggesting that it exists in solution as a dimer or tetramer. Purification of a second recombinant virus coding for the full length NGF-R is in progress.

537.13

NEURITOGENESIS IN CHROMAFFIN CELLS "IN VITRO":EFFECTS OF CENTRAL GLIA. J.A.Colombo,G.Perez*,R.Caccuri* and G.Dran*. Unidad de Neurobiologia Aplicada (CEMIC-CONICET),Buenos Aires, Argentina.

Adrenal medulla chromaffin cells have been proposed as a possible cellular replacement therapy in cases of human Parkinson's desease resistant to pharmacological treatment. Their reported ability to shift from an endocrine to a neural phenotype may or may not be critical for such proposed therapeutical role,depending on whether or not neural activity is required for their expected full clinical effects.Although their true role in such cases may still be a matter of debate, an analysis of factors capable of fostering a complete neural phenotype may prove to be of interest.Immature (PN 10) or adult rat adrenal medullae were enzymatically and mechanically dissociated. Cells were seeded on polylysine-coated coverslips and cultured in DMEM-horse serum(10%) or DMEM-F 12 media at 37°C and 95% air/CO2 atmosphere.The following groups were analyzed:NGF(2.5 S)100 ng/ml, with or without co-culture on top of a fetal, central glial carpet grown to confluence and physically separated from the chromaffin cells; controls with no glia or NGF were included. Infrequent spontaneous neuritogenesis was observed in control cultures.On the contrary, interaction between NGF and glia tended to support, or induce, a higher incidence of neuritic growth under present culture conditions.- This work was supported by the CONICET (Argentina).

538.1

COGRAFTS OF RETINA AND RETINAL PIGMENT EPITHELIUM TO ADULT RABBIT RETINA. R. Aramant, M. Seiler, A. Bergstrom* and A. R. Adolph. Eye Research Institute, Boston, MA 02114 and *Dept. of Ophthalmology, University of Lund, 22185 Lund, Sweden.

Retinal pigment epithelium (RPE) plays an important role in the development of the neural retina. We wanted to determine the influence of cografted RPE cells on the differentiation and survival of retinal grafts. Pigmented rabbit E16 embryos (total gestation time = 32 days; day of conception = E 0) were used as donors. Half of the retina was dissected free of RPE and other tissues, the other half was dissected together with the RPE sheet attached. The donor tissue was grafted to a retinal lesion site in 18 young adult rabbit hosts (Seiler et al., 1988, Soc.Neurosci. Abstr. 14:1276). Each host received a pure retinal graft in one eye and a retina/RPE cograft in the other.

Four weeks after transplantation, large grafts (1-2 mm dia.) were found in both experimental groups. Grafts (mostly fused with host retina) could be seen in the lesion site, the subretinal space or in the choroid. In the latter case, host RPE cells had been mixed with the donor tissue (indicated by remnants of pigment inside the graft). Grafts with RPE cells had a higher success rate and were mostly larger than pure retinal grafts. Neural retinal cells formed rosettes with all retinal layers around an outer limiting membrane irrespective of the presence or absence of grafted RPE cells. Grafted RPE cells often were organized in clusters or islands of cells inside the graft, partially without pigment, with a basal lamina around them. They were only occasionally found inside rosettes, in contact with the (still immature) photoreceptors. One-third of the retina/RPE cografts also contained grafted choroidal cells which apparently caused a lot of disorganization. After 8 weeks, grafts of both types appeared to be considerably smaller with less rosette formations which may indicate a degenerative process. Disrupted RPE cells which are not in contact with Bruch's membrane and the choroid might lose their influence on retinal cells. This possibility is being further investigated.

538.2

RETINAL TRANSPLANTATION AS A TOOL FOR THE STUDY OF RETINAL DEGENERATION. L. Qi Jiang and M. del Cerro. Department of Neurobiology and Anatomy, University of Rochester Medical School, Rochester, NY 14642.

Neural transplantation can be used as a tool to study neural degenerative diseases. Retinal transplantation, which provides the opportunity to implant the neural retina into a genetically defined intraocular environment, was used to study retinal degeneration in rd/rd mice. The site of the rd gene action remains undetermined; photoreceptor cell degeneration may be caused by intrinsic cellular factors with secondary changes in the intraocular environment. We designed a two-way transplant paradigm to test this possibility (ie.+/+ ->rd/rd and rd/rd ->+/+). C3H/Hej mice (rd/rd) and AKR/J mice (+/+) were used. The mice were homozygous at the rd locus (ie, rd/rd or +/+) and H-2k haplotype. Donors were postnatal day 0 (PND 0) and hosts were adult (8-9 weeks). Donor neural retina cells were transplanted into the subretinal space or the vitreous cavity of host eye. Serial sections of the eyes at post-transplantation day (PTD) 3, PTD 10-15 and PTD 30 were examined to search for successful grafts. Both groups of transplants had high survival rates at PTD 3. The grafts often showed photoreceptor differentiation mostly inside rosettes. A statistically significant difference appeared between the two groups of transplants at PTD 10-15. The survival rate of retinal grafts dramatically dropped in the +/+ ->rd/rd group. Histological analysis and immunohistochemistry for S-antigen (a specific marker for photoreceptor cells) indicated a regressive change in the photoreceptor cells of these grafts. In contrast, the grafts of the rd/rd ->+/+ group still retained a good survival rate at PTD 10-15. These grafts showed numerous rosettes containing photoreceptor cells with well-developed outer segments. The photoreceptor cells appeared to be the main population of the grafts. At PTD 30, although the graft survival rate of the rd/rd ->+/+ group was reduced, the photoreceptor cells (S-antigen positive) were still the majority of the surviving cells; this was not the case in the +/+ ->rd/rd group. These results suggest that intrinsic cellular defect may not be the only cause for retinal degeneration in rd/rd mice. Further studies are necessary to determine what interactions might play a role. In the meantime, retinal transplantation appears to be a promising tool for studying pathogenic mechanisms operating in genetic retinal degeneration. Supported by NEI grant # 05262.

538.3

SELECTIVITY OF CONNECTIONS MADE BY RETINAL TRANSPLANTS J.D. Radel, M.H. Hankin and R.D. Lund, Department of Neurobiology, Anatomy and Cell Science, University of Pittsburgh, Pittsburgh, PA 15261

Embryonic retinae transplanted to neonatal rat brains develop and integrate into functional pathways of the host visual system. In order to explore the functional potential of retinal transplants, we have examined here their detailed connections and how these are modified by transplant location.

Embryonic mouse retinae (E12-13: CD-1) were transplanted to the brains of neonatal rats (P1: Sprague-Dawley) from which one or both eyes had been removed. At 3-4 weeks of age, animals were fixed and sections of the brain stained with mouse neuron-specific antibodies to show the location of the transplant and its connections with the host brain.

The most extensive connections with host visual nuclei were made by transplants located on the dorsal midbrain. These showed projections to superior colliculus, the pretectum (including the nucleus of the optic tract and olivary pretectal nucleus), the outer shell of the dorsal lateral geniculate nucleus (dLGN) and the accessory optic nuclei. No projection was seen to the inner core of the dLGN, the ventral LGN (vLGN), the intergeniculate leaflet or to the suprachiasmatic nucleus (SCN). The same selectivity was found for grafts in other locations, although some regions were less predictably innervated. Even with transplants placed in the region of the optic chiasm, or on the adjacent optic tract, neither the SCN nor the vLGN was ever innervated.

It appears, therefore, that retinal transplants exhibit a selectivity of innervation that is not dictated by proximity to the target nucleus. This may perhaps be the product of a timing mismatch between the graft and host tissues, or may reflect the absence of a specific ganglion cell type(s). In addition to clarifying some of the rules governing the formation of specific anatomical connections between brain regions, this preparation may also be useful for examining the development of functional systems in the mammalian CNS. The presence of transplant-derived input to some visual nuclei and the absence of input to others (e.g., vLGN and SCN) provides a useful preparation for examining the substrates underlying the establishment of light-activated behaviors.

Supported by NIH grants EY05962 (JDR), NS26777 (MHH) and EY05283 (RDL).

538.4

MICROCIRCUITRY OF RETINAL TRANSPLANTS: ULTRASTRUCTURAL OBSERVATIONS. C. L. Zucker, M. Seiler, R. Aramant, A. R. Adolph. Eye Research Institute and Harvard Medical School, Boston, MA.

Successful retinal transplantation requires development of functional circuitry between neuronal elements within the graft and between graft and host. In the present study, we performed ultrastructural analysis of embryonic rat (E13, E15) retinas transplanted to the vitreal side of lesions made in adult retinas, after 7 to 19 weeks survival. At all survival times, donor retinas showed organization consistent with, but not identical to, normal retinas. Organizational centers (rosettes) frequently form interspersed within less organized regions. Photoreceptor (PR) cells typically form a ring with outer segments polarized toward a central core. Prominent connecting cilia with basal body complexes and inner segments were also within these cores. Rosette cores were delineated by "outer limiting membranes" of tight junctional complexes between PR and Muller cell processes. PR cell bodies, intermixed with amacrine cell (AC) bodies and "outer plexiform islands" surround rosette cores. Islands consist mainly of vesicle-filled PR synaptic terminals with one or more synaptic ribbons and several vesicle-filled post-synaptic elements probably of horizontal and bipolar cell origin. Juxtaposed to OPL-like islands, AC-to-AC synapses are sometimes found. Typically, an extensive region of conventional inner plexiform layer (IPL) is distal to the rosette. Within the grafts AC terminals, containing small electron-lucent vesicles (with or without peptide-containing large dense-cored vesicles), make conventional synapses onto AC and bipolar cell (BP) processes as well as cell bodies and unidentified terminals devoid of synaptic vesicles. AC synapses are also found in serial and reciprocal configurations. BP cell terminals make ribbon synapses with post-synaptic dyads composed of either two AC processes or one AC process and one process devoid of synaptic vesicles. In normal retinas, dyads typically contain one ganglion cell (GC) process which is devoid of vesicles and one AC process. The complexity of microcircuitry within retinal transplants suggests that such tissue has the potential circuitry necessary to perform visual information processing.

538.5

SURVIVAL AND MORPHOLOGICAL DEVELOPMENT OF TRANSPLANTED MAMMALIAN RETINAL GANGLION CELLS. A.M. Cooper*, I.D. Thompson* and P.M. Cordery* (SPON: Brain Research Association). University Laboratory of Physiology, Oxford University, OX1 3PT, England.

The significance of the normal retinal environment and the normal target tissue on the development of retinal ganglion cells is investigated by employing labelled neurone suspensions as transplants. Neonatal hamster retinal ganglion cells are retrogradely labelled from the superior colliculus with fluorescent latex microspheres prior to the main phase of ganglion cell death. The labelled retinal ganglion cells are dissociated into a cell suspension by gentle trituration and transplanted by injection through a micropipette into various target nuclei of host neonatal and adult animals. It is possible to relocate the transplanted, labelled cells in fixed tissue and to examine the survival of the neurones in relation to estimates of numbers transplanted. The technique also allows for the transplanted cells to be identified in in vitro preparations using fluorescence microscopy and for intracellular injection of a second tracer to reveal dendritic morphology. Future investigations will examine the connectivity of the transplanted cells both ultrastructurally and electrophysiologically.

538.6

TRANSPLANTATION OF INMATURE MOUSE RETINA INTO ADULT RAT EYES.

Coca del Cerro, Stephen M. Gallousis, Eliot Lazar and Manuel del Cerro. (SPON: A. Monjan). Dept. Neurobiology and Anatomy, Medical School, Rochester, NY. , U.S.A.

We have previously reported successful transplantation of developing mouse neural retina into the retinas of adult rat hosts, in the absence of immunosupresion (Neurosc. Abstr., 88). We have extended those studies since retinal xenografts offer unique opportunities to study host-donor cell interactions and may provide valuable information on the use of alternate sources of donor tissues. Donors were newborn (PND 0) C57BL/6J mice. Host were adult Fisher 344 albino rats. Dissociated and undissociated neural retinas were transplanted into the posterior pole of host eyes. No immunosuppressive procedures were used. Light and electron microscopical observation showed that the transplants survived and resulted in the differentiation of ONL cells, INL cells and a plexiform layer. The transplants were well accepted up to 17 days after transplantation, the latest time point observed in these series. The host reaction showed a few macrophages surrounding the transplants. This mild reaction is comparable to that observed in the intra-species retinal transplants at the same post-transplantation times. Although the transplants integrated closely with the host retinas, the mouse donor and rat host cells can be differentiated from each other by their cytological features under LM and EM. The observations show that short time retinal xenografts are feasible in the absence of immunosuppression. Our data show that by choosing judiciously the donor-host combination it is possible to achieve positive identification of the host and donor cells by means of intrinsic morphological or biochemical markers.

Supported by NEI grant 05262.

538.7

FETAL MONKEY RETINA TRANSPLANTED INTO ADULT RAT EYES. Manuel del Cerro, Coca del Cerro, and Jeffrey H. Kordower
Department of Neurobiology and Anatomy, University of Rochester, Medical School, Rochester, NY. 14642, and Department of Anatomy and Cell Biology, University of Illinois School of Medicine, Chicago, Illinois 60612

Transplantion of developing rodent retina into the retina of adult hosts has already been achieved successfully by us and others. However, the potential of developing primate retinal cells to serve as donor material in xenografts has not been established. We wanted to test the feasibility of this form of interspecies transplantation since it may offer new opportunities to study host-donor cell interactions as well as provide information concerning alternative sources of donor tissues. Donors were monkey fetuses (E 60 to E 90) of the species *Cebus Apella*. The fetuses were removed by Caesarean section. Hosts were adult Fisher 344 albino rats. Dissociated and undissociated neural retinal cells were transplanted into the posterior pole of host eyes. Depression of the host immune system (Cyclosporin A®;10 mg/ kg, daily) was maintained for the duration of the experiment. The host were allowed to survive for up to 33 days. Light and electron microscopic observations showed the differentiation within the transplants of photoreceptor cells (with inner segments and cilia attached to them), INL neurons and a synapse-rich plexiform layer. The transplants were well accepted throughout the survival period as there was no histologically demonstrable host reaction to the implants. The transplants closely integrated with the host retinas and actually overgrew the confines of the host retina, often expanding into the vitreal cavity. These observations demonstrate that non-human primate retinas are suitable donor tissue for transplantations as xenographs. These data offer exciting possibilities for further anatomo-functional studies of transplanted primate neuroretinal cells.
Supported by NEI grant 05262 and a generous anonymous donation.

TRANSPLANTATION III

539.1

IMPLANTATION OF GENETICALLY ENGINEERED ASTROCYTES INTO THE RAT BRAIN. V. Quinones#, H. M. Geller, M. Poltorak* and W. J. Freed*. (Sponsor: T. McGuire). Dept. of Pharmacology, UMDNJ-Robert Wood Johnson Medical School, #The Graduate School, Rutgers University, Piscataway, NJ 08854 and *NIMH Neurosciences Center at St. Elizabeth's, Washington, DC 20032

A7 cells are an immortal astrocyte cell line made by inserting the gene for SV40 large T antigen into primary astrocytes from the Sprague-Dawley rat via retroviral-mediated gene transfer (Geller and Dubois-Dalcq, 1988). This cell line has certain astrocytic properties, such as the expression and secretion of PDGF, expression of the p185 phosphoprotein product of the *neu* oncogene, and the ability to support the growth of dissociated embryonic neurons *in vitro*. A suspension of A7 cells was labelled with bisbenzimide and implanted into the stratum parenchyma of naive Sprague-Dawley host animals. Animals were sacrificed after 2-8 weeks of survival and their brains were examined using H & E staining as well as indirect immunofluorescence for examination of cell survival and the effect of these cells on the host brain tissue. Implanted A7 cells were identified by the presence of bisbenzimide labelling as well as with a monoclonal antibody against large T antigen which brightly stains the nuclei of the implanted cells. The grafted cells did not produce neoplastic growth and only some of them survived. They did not induce evident immunological reactions within the host brain as measured by the presence of $OX6^+$, $W3/25^-$, $W3/13^+$ and $OX8^+$ cell infiltrations. Distinct laminin-positive immunoreactivity was closely associated with the implanted cells, which retained their antigenic phenotype, including the surface expression of N-CAM. In animals examined after 6 and 8 weeks, the Thy-1.1 immunoreactivity within the surrounding host brain seemed to fill the area of grafted tissue. The present data, together with *in vitro* observations that the A7 cell line promotes neuritic outgrowth, suggests that these cells may be useful in models of restoration of function by intracerebral implantation. Supported in part by NIH P01 NS 21469.

539.2

EXPRESSION OF HUMAN GROWTH HORMONE BY INTRACEREBRAL TRANSPLANTS OF GENETICALLY MODIFIED FIBROBLASTS
L.C. Doering and P.L. Chang*. Departments of Biomedical Sciences (Anatomy) & Pediatrics, McMaster University, Hamilton, Ontario, CANADA L8N 3Z5.

Cells can be genetically engineered and transplanted into neural and non-neural sites to secrete specific products (M.B. Rosenberg et al., 1988, Science 242:1575-1578; R.F. Selden et al., 1987, Science 236:714-718).

We have examined the feasibility of a non-retroviral system to genetically modify primary fibroblasts for the delivery of a gene product to the central nervous system.

Primary fibroblasts from Wistar rats were transfected by calcium phosphate precipitation with the plasmid pNMG3, constructed to encode for the neomycin resistant and human growth hormone (hGH) genes.

Fibroblasts were selected in vitro for resistance to the neomycin analog G418 and for a high level of hGH secretion. These cell clones were subsequently isolated, grown to confluency and then implanted into the cortex of adult Wistar rats with a Hamilton syringe. After 3-4 weeks the fibroblasts were examined by immunocytochemical methods with antibodies to hGH and fibronectin. Characteristic patterns of hGH immunostaining in the cytoplasm indicated that the fibroblasts had survived and expressed the reporter gene product. These experiments illustrate the potential of this system as a model to study gene replacement therapy in the CNS.

539.3

RETROVIRALLY-INSERTED ß-GALACTOSIDASE GENES AS MARKERS FOR DONOR CELLS IN NEURAL GRAFTING. J.N. Kott, L.E. Westrum, C.E. Ogburn*, T.J. Kavanagh*, and G.M. Martin*, Depts. of Neurological Surgery, Biological Structure, Pathology, and Medicine, University of Washington, Seattle, WA 98195.

Full interpretation of many neural grafting experiments requires discrimination between host and donor cells after a lengthy survival period. To aid in such discrimination we are incubating cell suspensions or pieces of fetal rat olfactory bulbs (OB) with a replication-incompetent retrovirus containing a bacterial ß-galactosidase (ß-gal) gene. These labeled cells or pieces are subsequently grafted into the OBs of adult and neonatal rats. After varying survival periods up to several weeks, host tissue is fixed and histochemically processed for ß-gal reactivity. In initial experiments we have found dense cytoplasmic reaction product in cells of varying morphology in the area of grafts. ß-gal positive cells include: round, spindle, pleomorphic and pyramidal-like shapes of up to 20 um in length. Small glia-like cells are also seen near blood vessels and pia.

Work is in progress to further increase the proportion of donor cells expressing ß-gal and to characterize the ultrastructural distribution of reaction product. This retroviral method promises to be as attractive in marking grafted donor cells as it is in studies of cell lineage. (Supported by Grants NS09678 and AG05136. LEW is an affiliate of the CDMRC, and GM is Director of ADRC).

539.4

AUTOGRAFTS OF PRIMARY FIBROBLASTS TO THE RAT BRAIN REMAIN AT A CONSTANT VOLUME OVER TIME. B.L. Firestein, A.M. Fagan and F.H. Gage. Dept. of Neurosciences, UCSD, La Jolla, CA 92093.

This study investigated survival of primary fibroblast autografts in the rat brain to assess the potential of these cells as candidates for grafting after genetic modification. Two variables were addressed: 1) time in culture, and 2) post-operative survival time in the brain. Skin biopsies were taken from the perineum of each rat, and pure fibroblast cultures were grown to confluency and harvested at a resting state. First and fourth passage cells were suspended in sterile saline (1×10^5 cells/µl) and injected bilaterally into the caudate. Rats were sacrificed at 3 and 8 weeks post-operatively. Frozen coronal sections were cut serially at 40 µm and immunostained with OX-42, to analyze wound response and ∝Fibronectin, to localize grafted fibroblasts. An image analyzer was used to compute graft volumes (in mm^3): Pass 1, 3 wk = 7.29± 0.758; Pass 1, 8 wk = 7.258 ± 1.59; Pass 4, 3 wk = 6.348 ± 1.735; Pass 4, 8 wk = 5.623 ± 1.106. No significant differences were observed in graft volume as a function of time in culture or survival time in the brain ($p < 0.79$). These data suggest that 1) fibroblast autografts survive in the brain for up to 8 weeks, 2) fibroblasts do not tumor in the brain over time, however they may grow and die, and 3) these cells do not migrate. These findings suggest that these cells may be suitable for grafting after genetic modification. Studies that address optimal cell density for grafting and cell turnover are currently in progress.

539.5

GLIAL FIBRILLARY ACIDIC PROTEIN (GFAP) IMMUNOCYTOCHEMISTRY OF FETAL TRANSPLANTS IN TRAUMATIC BRAIN INJURY. H. Soares and T. McIntosh. Dept. Surgery, Univ. of Conn. Health Ctr., Farmington, CT 06032.

Lateral fluid percussion (FP) traumatic brain injury of adult male rats (300-400g) results in reactive gliosis within injured parietal motor cortex. A cavity, surrounded by a well defined glia limitans develops at the injury site two to four weeks postinjury. Previous studies have demonstrated that successful E16 fetal parietal cortex transplantation into the cavity is associated with improvements in neurological function. However, connection of fetal tissue was dependent upon the extent of glial scarring (i.e., time dependent). This study further characterizes post-traumatic fetal transplants using GFAP immunocytochemistry, acetylcholinesterase, and toluidine blue. Animals received fetal (E16) cortical tissue 2 days, 1 week, 2 weeks, and 4 weeks after FP traumatic injury and were sacrificed 4 weeks post-transplant. Transplants performed at 2 days and 1 week postinjury survived and showed optimal connectivity compared to later timepoints. Successful transplants exhibited continuous connections, dense neuronal populations, extensive vascularization along transplant/host interface, and fewer positive GFAP cells than unsuccessful transplants. There were no successful transplants observed at the 4 week postinjury timepoint. Unsuccessful transplants contained dense populations of positive GFAP cells located along the transplant perimeter, and numerous positive GFAP cells were observed in corpus collosum and both ipsilateral and contralateral external capsules. These observations support the hypothesis of a "critical time window" for successful postinjury fetal transplantation in traumatic cases involving reactive gliosis.
Supported in part by VA Merit Review 74R and NIH R01-NS26818

539.6

INTEGRATION OF GRAFTED NEURAL TISSUE WITH THE HOST BRAIN. K. Shigematsu*, H. Kamo*, I. Akiguchi*, H. Kimura* and J. Kimura* (SPON: N. Mizuno). Dept. of Neurology, Kyoto Univ., Kyoto and Shiga Univ. of Medical Sci., Shiga, JAPAN

We studied axonal connectivity between transplants and the host brain and the associated tissue reactions immunohistochemically in rats with or without cytosine arabinoside (Ara-C) treatment.

The brainstem cell suspensions were prepared from 14 to 16 day old Wistar rat fetuses. After incubation with or without PHA, the cells were transplanted stereotaxically into the lateral ventricles or the striatum of 200g–250g Wistar rats, pretreated by 6-OHDA to destroy nigra-striatal pathway. At various times up to 3 months after transplantation, rats were sacrificed under deep anesthesia to examine serial cryostat sections of the brains. In 10 rats, PHA was injected inside or outside of the transplants two days before sacrifice to trace the axonal connectivity between the grafts and the host brain. PHA, tyrosine hydroxylase or serotonin immunohistochemistry demonstrated neurons and their processes successfully grafted in the ventricle or the striatum but no axonal elongation from the graft into the host neuropil. Observation at the graft-host border revealed numerous reactive astrocytes, microglia and neovascularization by GFA, ox6 and ox18, and laminin and type IV collagen immunohistochemistry. Treatment with Ara-C for 7 days decreased glial reaction allowing some neuronal processes from the graft crossing the border extending into the host neuropil. Antimitotic agents may promote neurite growth extending into neuropil by inhibiting glial proliferation.

539.7

TIMING AND PATTERNS OF ASTROCYTE MIGRATION FROM XENOGRAFTS OF CORTEX AND CORPUS CALLOSUM. H.F. Zhou, L. Lee* and R.D. Lund, Dept. Neurobiol., Anat. and Cell Sci., Univ. Pittsburgh, Pittsburgh, PA 15261

The timing, patterns and pathways of astrocyte migration were investigated *in vivo* after transplantation of CD-1 mouse cerebral cortex (E13-14) or corpus callosum (P2-3) to the cortex, the subcortical white matter or the hippocampus of Sprague-Dawley albino rats (P1). Host brains were fixed with zinc-aldehyde at post-transplantation day (PTD) 4-30. A monoclonal antibody to a mouse astrocyte surface antigen (M2) was used to identify the location of the grafts and the migrated astrocytes from the grafts. Over 90% of cells stained with anti-M2 also stained with anti-GFAP, confirming that labeled cells were astrocytes. Within the host cortex, astrocytes from cortical grafts began migration at PTD 7. Over the next 4 days, the most distant displaced donor cells were found progressively further away from the grafts (60, 300, 650 and 900 um), suggesting an average migration rate of about 220 um/day. After that, the migration slowed to 40 um/day, and stopped at about PTD 16 at a distance of 1100 um. Astrocytes from callosal grafts migrated into both the gray matter and the white matter. The longest migration distance observed in the white matter was 5 mm. In a callosal graft implanted near the host corpus callosum, a few anti-M2 stained cells were found in the white matter of the contralateral hemisphere having crossed the midline and migrated as much as 900 um.

The patterns of astrocyte migration differed depending on the location of the transplant. Donor astrocytes which had been implanted into cortex migrated toward the surface of host cortex, sometimes in several radial lines. However, migrating astrocytes implanted in the hippocampus showed a more variable distribution pattern. In some cases, the distribution of labeled cells closely followed that of the neuronal cell layer of host hippocampus, while in others, donor cells distributed randomly around grafts. Some cells were also seen in the cortex and midbrain. No labeled cells were found along the injection pathway.

The results demonstrate the timing of astrocyte migration from grafts and a relationship between the patterns of astrocyte migration and the locations of the grafts. (Supported by NIH grant EY05308).

539.8

MIGRATION OF GRAFTED NEONATAL ASTROCYTES IN NEONATAL AND ADULT HOSTS. J. D. Hatton and H. S. U.* Division of Neurosurgery, UC San Diego and Veterans Administration Hospital, La Jolla, CA 92093

The migration of neonatal rat cortical astrocytes transplanted into the brains of adult hosts has previously been examined. However, the effects of different sources of donor astrocytes, and of developmental stage of the recipient on this migration is unknown. Therefore, we grafted Fast Blue labelled astrocytes from 1-3 day old rat cerebral cortex, hippocampus and hypothalamus into neonatal and adult brains by injection with a Hamilton syringe. Dorsal or basal forebrains were targeted in the neonatal hosts, while cortex, hippocampus and hypothalamus were targeted in adult hosts.

Transplanted astrocytes dervied from the neonatal cerebral cortex migrated extensively throughout the adult brain, using the ventricular walls, glial limitans, vasculature and fiber bundles to guide their movements. However, hypothalamic astrocytes homografted into the adult hypothalamus migrated only within the hypothalamus and basal glial limitans. Likewise, homografted hippocampal astrocytes migrated throughout the adult hippocampus and ventral cerebral cortex only. In both cases, migration routes appeared to be random or unguided. In neonatal hosts, some unguided migration was noted in grafts in all areas. Most migration occurred along the vasculature of the developing brain, and was less extensive than in the adult hosts. These results suggest that the migration patterns of grafted astrocytes are dependent on source organs, and that the scaffolding that defines migration routes in the adult brain are not yet well defined in the neonate.

539.9

IMPLANTATION OF IMMATURE ASTROCYTES INTO THE CONTUSED SPINAL CORD: CHRONIC EFFECTS ON FUNCTIONAL DEFICIT AND HISTOPATHOLOGY. J. Wrathall, R. Pettegrew, M. Castro* and L. Verma*. Dept. of Anatomy & Cell Biology, Georgetown Univ., Washington, D.C. 20007.

We have previously shown that astrocytes cultured from the embryonic spinal cord can be implanted into the normal or contused adult rat spinal cord and survive for at least 1 week. In order to examine the long-term effects of such implants, groups of rats (n = 20) were subjected to a standardized mild contusive injury and implanted either immediately (Immediate) or 1 week later (Delayed) with a suspension of 2-5 X 10^5 viable astrocytes. Controls were contused but not implanted. Rats were maintained for up to 6 months after injury/implantation with functional deficits assessed by our standard battery of behavioral tests at 1 day, weekly through 4 weeks and monthly thereafter. They were then perfused for histopathology or immunocytochemical analysis of the injury sites. Functional deficits appeared similar for control and implanted groups through 2 months after injury. However, at 4 and 6 months the average inclined plane score of the delayed implant group was significantly better than that of other groups. In addition, at 6 months the average percentage of errors in grid walking appeared less. The implanted injury sites exhibited enhanced GFAP and vimentin immunoreactivity. The results suggest that implants of immature astrocytes may have long-term effects on the injured spinal cord.

539.10

MIGRATION AND SURVIVAL OF OLIGODENDROCYTES IMPLANTED INTO A FOCALLY-DEMYELINATED SPINAL CORD. J. Serratosa* and J.J. Lopez-Lozano. Lab. of Neurobiology. Dept. Neurology and Exp. Surgery. Clinica Puerta de Hierro. Univ. Autonoma. 28035-Madrid. Spain.

As a previous step to study the influence of enriched populations of glial cells on the survival and plasticity of transplanted neurons, we decided to study wether a population of neonatal oligodendrocytes (OL) was able to survive and develop into mature cells after being implanted into a focally demyelinated spinal cord. Fourteen to 21-day-old Wistar-Lewis rats were ether anesthetized, decapitated and their spinal cord were dispersed by an enzymatic-mechanical procedure. OL were obtained by a percoll density gradient and were injected into a focally-demyelinated spinal cord induced by an injection of 1% lysolecithin one week before. At intervals of one to eight weeks, animals were reanesthetized and intracardially perfused with apropiate fixatives. The spinal cords were removed and the lesion morphologically analyzed by LM and EM techniques as well as by immunocytochemistry of galactocerebroside (GC), myelin basic protein (MBP) and glial fibrillary acidic protein (GFAP). Preliminary results show that implanted cells seem to migrate from the implantation site to the lesion and that implanted-OL express myelin markers. Transplanted animals show an increase in the number of OL and a higher myelination rate as compared to saline injected-control animals . Those results seem to indicate that transplanted OL survive and produce myelin in the host's demyelinated spinal cord and that this can be a useful model to develop new strategies in the study of demyelinating diseases. (Supported by FIS 87/875, 88/1547 and Severo Ochoa-Ferrer Foundation award to JJLL).

539.11

STUDY OF NEURAL REGENERATION AFTER TRANSPLANTATION: USE OF PHASEOLUS VULGARIS LEUCOAGGLUTININ AS A CELL MARKER AND THE EFFECT OF DIHYDROERGOTOXINE MESYLATE. H. Kamo*, K. Shigematsu*, I. Akiguchi*, H. Kimura* and J. Kimura* (SPON: H. Ando). Dept. of Neurology, Kyoto Univ., Kyoto and Shiga Univ. of Medical Science, Shiga, Japan.

We present a Phaseolus vulgaris leucoagglutinin (PHA) marking method for delineating transplanted cells and their sprouting processes. Cultured neural tissues or cell suspension prepared from embryonic rat brains were marked by brief incubation with PHA and then transplanted into the striatum or the cortex of nonimmunosuppressed young adult rats. At various time intervals (up to 2 months), the animals were sacrificed under deep anesthesia and serial cryostat sections were examined. Transplanted neurons and their presumably regenerated processes were successfully demonstrated by PHA immunohistochemistry. Thus, PHA can be used as a marker for delineating transplanted cells and their sprouting processes. PHA has the following merits as a marker in neural transplantation: First, PHA is readily taken up by neurons just prior to transplantation. Second, it is retained for a more prolonged period than HRP. Third, it is transported anterogradely and reveals the post-transplantation growth of the neuronal processes.

Neural transplantation with PHA marking can be also applied as a model to study neural regeneration or evaluate in vivo effects of various types of neurologically active substances on the neurite growth. We used the method to study the effect of dihydroergotoxine mesylate on the transplanted neurons.

539.12

LAMININ INJECTIONS GUIDE AND PROMOTE FIBER GROWTH OF TRANSPLANTED AND REGENERATING NEURONS IN ADULT BUT NOT IN AGED BRAIN. F. Garcia-Hernandez, F.C. Zhou, and E.C. Azmitia. Dept. Biology, New York University, NY, NY 10003.

Laminin directs outgrowth of transplanted (TP) neurons into adult hippocampus (HIP) (Zhou and Azmitia, J. Chem. Neuroanat., 1:133, 1988). Does laminin guide fetal neurites into the aged and regenerating sprouts in the adult brain? For TP studies, adult and aged S-D male rats (3 and 25 months) received a cell suspension of fetal 5-HT neurons bilaterally into the HIP. A laminin tract was made 0.5 mm lateral from the TP site. Adult female rats were lesioned with 5,7-DHT (3 ug/250 nl) into the MFB to induce sprouting, and 14 days later, a laminin tract was made from the lesion site toward the ventral hypothalamus. 5-HT immunocytochemistry was done after two weeks. Dense and straight fibers from the grafted tissue were seen within the laminin tract of adult, but not aged animals or vehicle tracts. 5,7-DHT-induced sprouts elongated toward the laminin tract. The absence of the elongation in the aged brain is due to factors unknown to us. Supported by NSF BNS-8812892 and NIH BRSG 2 S07 RR 07062.

539.13

ANALYSIS OF INTRASPINAL NITROCELLULOSE FILTER IMPLANTS. F. J. Liuzzi and B. Tedeschi*, Eastern Virginia Medical School, Norfolk, VA 23501.

Implantations into the mammalian spinal cord of neonatal astrocyte-coated filters have been reported to diminish host response to injury and provide a substrate for axonal growth (Silver, CNS Trauma Symp., 1987). The aim of this study was to evaluate, using EM, the influence of filter implants on host adult rat spinal cords. Following avulsion of L3,4 and 5 dorsal roots one of three types of filters were implanted within a slit along the dorsolateral sulcus after which the roots were tucked medially along the filter. Survival times were up to 2 months. The three filter types were: 1) uncoated; 2) polylysine-collagen coated; and 3) astrocyte-coated (cells harvested at either E15 or at birth). The first two types of filters evoked similar host responses. A connective tissue matrix (CTM) formed around the filters as did a new glia limitans. In animals in which the dorsal roots were labelled with HRP, regenerating axons were seen in CTM around the filters and occasionally within the cord. At the EM level, Schwann cell myelinated axons were observed within the CTM but not on or immediately adjacent to the filters. Filters coated with astrocytes harvested at E15, were also surrounded by a CTM and were devoid of surviving astrocytes. Implants with newborn astrocytes are currently being analyzed. Supported by NIH Grant NS24309, SCRF (FJL), and Institutional Grants (FJL, BWT).

539.14

INTRACEREBRAL IMPLANTATION OF IONOGENIC MATRICES. S. Woerly, R. Marchand, C. Lavallée* and R. Guidoin*. Lab., Neurobiol., Hôp. Enfant-Jésus, Centre de rech. Sci. & génie des macromol., Univ. Laval, Lab. Biomat., Hôp. St-François d'Assise, Québec.

The introduction of an extracellular matrix-like architecture,an artificial and permanent scaffolding structures for brain wounds, may assist tissue ingrowth and regeneration. Synthetic hydrogels of poly (glyceryl methacrylate) (poly GMA) are well tolerated by brain tissue and, depending on pore characteristics, allowed ingrowth of tissue (Woerly et al. Neurosci. Abstr. 1988). Here, the effect of ionogenic groups incorporated into polyGMA hydrogels on the reactivity of the lesioned neural tissue was studied since regenerating axons may respond to charges *in vivo* and *in vitro*. Charged polyGMA were obtained by polymerization of a monomer solution containing either basic groups with diethylamino-ethyl methacrylate (DEAEMA) or acid groups with methacrylic acid (MAA). Collagen was adsorbed into the polymers to give the necessary bioadhesivity. SEM showed matrices of polyGMA-DEAEMA with pores of 10µm while matrices containing MAA had heterogeneous porosity. Samples were implanted into the parietal cortex of adult rats, and after 3 months, the brains were examined in histology (cresyl, PAS, Bodian) and for GFAP. The implants caused a variable astrocytic reaction. Astrocytic processes contacted the gels and some entered. Extracellular material (reticulin and PAS substances), capillaries and heterogeneous cells accumulated at the brain-implant interface and reticulin was deposited into the polymers. Nerve fibers grew marginally in the tissue, some crossing the interface and a few projecting onto or into the matrices. Matrices carrying charged groups influence positively the brain wound healing (MRC, FRSQ & FCAR).

539.15

IMMUNE REACTIONS IN HOST RATS AGAINST INTRACEREBRAL GRAFTS OF MOUSE HIPPOCAMPUS AND MOUSE GLIAL CELL CULTURES. E.B. Pedersen[1]*, B.R. Finsen[1]*, M. Hokland[2]* and J. Zimmer[1]*. (SPON: M.J. West). PharmaBiotec, [1]Inst. of Neurobiology and [2]Inst. of Microbiology, Univ. Aarhus, Aarhus, Denmark.

Rejection of fetal C57-mouse hippocampal xenografts inserted bilaterally into the hippocampal region of adult Kyoto rats is usually completed after 5 weeks. The cellular infiltration of the grafts increased up to 3 weeks postgrafting. The infiltrate consisted mainly of macrophages and T-cells of the Ts/c and NK-cell fenotype. Antibodies cytotoxic to mouse spleen cells were found after 1 week. Within 1 week astrocytes at the graft site became highly reactive for glial fibrillary acidic protein (GFAP). Two weeks later this reactivity had decreased within the grafts, while it persisted in the host. Microglia-like cells, expressing class I and II MHC as well as leucocyte common antigen (LCA) were found both within and around the grafts. So far an astrocytic coexpression of GFAP and MHC antigen has not been observed.

The rejection of grafts of carbocyanine-labelled mouse astroglia cultures is also being investigated. Surviving, carbocyanine-labelled GFAP-reactive xenografts have been found up to 5 weeks postgrafting. Cytotoxic antibodies were found 1 week postgrafting. γ-interferon stimulation of astroglial cultures is currently being employed to increase the expression of class I and II MHC antigens prior to grafting.

539.16

CHANGES IN PERIPHERAL NERVE MHC CLASS II ANTIGENS FOLLOWING TRAUMA. T. E. Trumble*, J. Stanislaw*, S. Jacobson*, N. Troiano* (SPON: C. Duncan). Dept. of Orthopaedics and Rehabilitation, Yale Univ. School of Med., New Haven, CT 06510.

As a prelude to the transplantation of peripheral nerve tissue, human peripheral nerve tissue samples were analyzed for the presence of MHC Class II antigens and a mouse model was used to evaluate the effect of trauma on the concentration of MHC Class II antigens. (Methods) Part I: 7 autopsy and 5 operating room (OR) specimens were obtained from individuals without systemic disease. The specimens were frozen with liquid nitrogen, OCT imbedded and stained with equine anti-mouse IgG biotinylated secondary Ab and avidin DH-horse radish peroxidase H complex followed by 3-3 diaminobenzidine. Part II: 10 C_3H-hej mice underwent division of the sciatic nerve. Three weeks after surgery the nerve distal to the transsection site and the contralateral nerve were harvested, fixed as above and stained with anti-lack murine Ab plus goat anti-mouse IgG2b secondary Ab. (Results): MHC Class II antigens are present in human peripheral nerves and trauma increases the concentration of MHC Class II antigens in the mouse model. Controlling the rejection response to MHC Class II antigens may be important in peripheral nerve transplantation.

539.17

METABOLIC PROPERTIES OF GRAFTED NEURONS AND POTENTIAL RELATIONSHIP TO THE BLOOD-BRAIN BARRIER (BBB). J.M. Rosenstein, N. More and J.M. Krum, Dept. of Anatomy, The George Washington University, Washington, D.C. 20037.

Depending on placement, the BBB to protein in neural grafts can be variably permeable. Being exposed to the circulation, graft neuronal metabolic activity could be altered from the normal. Young adult rats received an IVT or parenchymal (IP) graft of fetal neocortex from E17-19 donors. Vibratome sections were examined for anti-Neuron Specific Enolase (NSE), a glycolytic enzyme and reliable predictor of the metabolic and developmental state of neurons. Adjacent sections were stained with rat anti-serum albumin (SA), to determine endogenous protein permeability while other sections were stained for cytochrome oxidase (CO) an indicator of metabolic activity. Early grafts had weak NSE activity and were entirely permeable to SA. By 6 weeks IVT grafts had highly variable anti-NSE staining; only a few grafted neurons were heavily stained and some not at all particularly in those regions that were stained for anti-SA; no laminar patterns were seen. CO staining in IVT grafts was weak. In IP grafts, occasional SA exudation was present at interface regions. NSE staining was again variable; neuropil reactivity was reduced from surrounding normal cortex. These studies, in which graft metabolism can be indirectly correlated with BBB integrity show that only a slight portion of grafted neurons have a normal NSE activity in unlesioned hosts. (NS-17468).

539.18

NEUROVASCULAR REORGANIZATION AND PLASTICITY FOLLOWING FETAL HYPOTHALAMIC TRANSPLANTATION INTO THE MAMMALIAN THIRD CEREBRAL VENTRICLE. W. Wu*, D.E. Scott and E.S. Miller, Department of Anatomy and Cell Biology, Eastern Virginia Medical School, Norfolk, VA 23501.

This investigation deals with neurovascular organization of fetal neural grafts in the third cerebral ventricle of DI host rats. Fine capillary networks were extant in grafts within the first week following transplantation. Most capillaries of fetal grafts were unfenestrated. Limited numbers of fenestrated capillaries were found at the interface between the graft and underlying host median eminence. Axon profiles filled with dense core vesicles were observed to terminate upon perivascular spaces of fenestrated capillaries. Perivascular spaces of the graft were narrowed and less extensive than those seen in the host median eminence. Vessels that grew distalward into the parenchyma of overlying fetal grafts lost their fenestrated characteristics, and tight junctions were observed between adjoining endothelial cells. These data suggest that the barrier properties of host vessels that fuse with primordial endothelial elements of fetal grafts are essentially different. Those that are found deep within graft parenchyma are phenotypically expressed as normal cerebral capillaries with an apparent blood brain barrier. However, those that are found at the interface between the graft and the host appear to be of the "leaky" variety and may not possess a true blood brain barrier. Supported by NSF Grant 8709687.

539.19

INTRACEREBRAL CELL SUSPENSIONS AND THE BLOOD-BRAIN BARRIER. P. Ebert*, R. Broadwell, A. Wolf, & G. Bergey (SPON: M. Salcman). Div. Neurosurg., Univ. of Maryland, Balto., MD 21201.

Cell suspensions of 1 day neonatal hippocampal neurons, type I astrocytes, and oligos, a fibrosarcoma model dog tumor and adult rat anterior pituitary gonadotrophs ($2x10^5$-$1x10^6$ cells in 10-20ul) were delivered into the striatum of adult athymic mice. Donor brains and pituitary glands were homogenized, passed through nylon filters, and cultured for 9da. Oligos were removed by shaking overnight and re-cultured for 7da; astrocytes stained positively for GFAP. All cells were trypsinized and washed in saline. Hosts were fixed 3-21da. post-operatively. Vascularization of cell suspensions with host endothelia perfused with host blood was partial at 3da and complete at 7da. Peroxidase delivered intravenously to the hosts at 10da and longer demonstrated the presence of a blood-brain barrier (BBB) to blood-borne protein in neuronal and glial suspensions, but not in tumor and gonadotroph cell suspensions. Endothelia in neuronal and glial suspensions were non-fenestrated and equivalent to typical BBB endothelia, whereas those in tumor and gonadotroph suspensions exhibited open junctions and/or fenestrae. The results suggest that the morphological characteristics of host endothelial cells supplying cell suspensions are determined by the donor cells. Supported by NINDS grant#NS18030.

539.20

TISSUE OXYGENATION IN SPINAL TRANSPLANTS: A REGULATED VARIABLE? B.T. Stokes, Dept. Physiology, The Ohio St Univ. and P.J. Reier, Dept. Neurosci., Univ. Florida.

Little is known about the role of the cellular microenvironment in transplant mediated repair in the central nervous system. This is in spite of the multitude of evidence that suggests that anatomical integration of host and graft tissue is critically dependent on a series of growth factors, developmental influences, and metabolic changes that signify the success of the grafting process. We report here on the potential role of tissue oxygenation (P_TO_2) in the maintenance and growth of fetal spinal transplants for the first three months after graft placement in an adult host aspiration-injured spinal cord.

In anesthetized rats with a L1-L2 laminectomy, we examined the lumbar spinal cord P_TO_2 of animals transplanted with fetal spinal cord up to three months previously. Frequency histograms were constructed from samples taken at different ages, depths into the spinal neuropil, and anatomical locations of host/graft zones. Correlative light and EM histological evaluations were also done on these spinal regions.

Within the fetal grafts, the P_TO_2 was like that found in the fetal cord in situ (<10 mm Hg). Such low values extend to the adjacent host tissue in an age dependent fashion. As grafts develop, their P_TO_2 and that of the adjacent host tissue approaches that of the normal adult spinal cord (20-30 mm Hg). Less well developed grafts, cavity sites in graft tissue, gliotic host/graft interfaces and untransplanted cavity sites all have P_TO_2 tensions in excess of the normal adult range (>35 mm Hg). The oxygen probe can therefore be used in a descriptive way to delineate periods of rapid graft development, graft/host fusion, and the occurrence of cavitation sites that may be incompatible with adequate neuronal survival. (Supported by NIH grant NS10165).

NEUROENDOCRINE REGULATION II

540.1

DOPAMINERGIC AND β-ADRENERGIC CONTROL OF α-MSH SECRETION DURING STRESS. S.E. Lindley*, K.J. Lookingland and K.E. Moore, Dept. of Pharmacology/Toxicology, Michigan State University, East Lansing, MI 48824.

Secretion of α-MSH from the intermediate lobe (IL) of the pituitary can be stimulated by epinephrine released from adrenal medulla and inhibited by dopamine released from tuberohypophysial dopaminergic (THDA) neurons terminating in the IL. Epinephrine has been reported to be responsible for the stress-induced increase in α-MSH secretion, but restraint stress also decreases the activity of THDA neurons projecting to IL (Lookingland et al., 1987, Soc. Neurosci. Abst. 13:418). The purpose of this study was to determine the role of the decrease in THDA activity in the stress-induced increase in α-MSH. In control animals, the dopaminergic antagonist haloperidol and the β_2 adrenergic agonist metaproterenol had an additive effect on the secretion of α-MSH, indicating a reduction in dopaminergic inhibitory tone enhances the effect of β-adrenergic stimulation. In restrained animals, pretreatment with the dopaminergic agonist apomorphine prevented the stress-induced increase in α-MSH secretion, indicating that maintenance of dopaminergic "tone" prevents the α-MSH response to stress. These results indicate that a decrease in THDA inhibitory tone is necessary for full expression of the stress-induced increase in α-MSH secretion. (Supported by NIH grant NS15911.)

540.2

INCERTOHYPOTHALAMIC A13 DOPAMINE (IHDA) NEURONS: EFFECT OF GONADAL STEROIDS ON TYROSINE HYDROXYLASE (TH) IMMUNOREACTIVITY. M.K. Sanghera, S. Grady*, W. Smith, D.J. Woodward and J.C. Porter. Depts. of Psychiat., Cell Biology and Ob/Gyn. U.T. Southwestern Med. Cntr., Dallas, Tx 75235.

Recent data from our laboratory indicate that IHDA neurons influence luteinizing hormone and prolactin levels in the intact female, and gonadectomized (GNX) male and female rats. In this study the effects of gonadectomy and steroid replacement on IHDA neurons was assessed by immunocytochemistry using an antibody against TH. A computer graphic system interfaced to a microscope was used to count, and measure the diameters of TH-positive neurons and display the data in the 3-dimensional space of the nucleus. In GNX males, there was a significant decrease in the number of TH-positive neurons compared to intact males ($p < 0.03$). In GNX females, there was a decrease in immunoreactivity throughout the nucleus but this was not indicated by a change in the total number of TH-positive neurons. Hormone replacement in both sexes restored TH immunoreactivity (cell number and size) to intact levels. These data suggest that TH expression in ICDA neurons is stimulated by gonadal steroids in male and female rats. (Supported by NINDS grants NS-24290 & NS-25321).

540.3

IN VIVO DOPAMINE (DA) RELEASE FROM THE CAUDATE NUCLEUS (CN) OF FEMALE RATS IN RESPONSE TO L-DOPA VARIES WITH THE ESTRUS CYCLE. D. E. Dluzen and V. D. Ramirez. Department of Physiology & Biophysics, University of Illinois, Urbana, Ill.

Intact female rats (N=5) with regular four day estrous cycles were implanted with a push-pull cannula directed at the CN and were perfused on each day of their estrus cycle. Perfusate samples (6-8 ul/min) were collected at 15 min intervals and assayed for DA using HPLC-EC. Following a one hour basal collection period, L-DOPA, diluted in perfusion medium, was infused directly into the CN through the push side of the cannula at two increasing doses (1 and 10 uM) during collection intervals 5 and 9. Maximal basal (mean DA release - pg/15 min) and L-DOPA stimulated DA release (area under curve - pg/75 min) were consistently obtained for animals perfused during proestrus and minimal levels at estrus.

	MEAN LEVELS (pg)			
	Estrus	Diestrus I	Diestrus II	Proestrus
Basal	27.5	29.8	39.8	47.1
1 uM L-DOPA	1507	1788	1282	2902
10 uM L-DOPA	4931	6345	6324	11514

These results demonstrate that physiological fluctuations in hormonal levels which occur during the estrous cycle in the female rat modulate both basal and L-DOPA stimulated DA release from the CN of freely behaving animals, indicating the importance of the endocrine milieu in the function of the nigrostriatal dopaminergic system.

540.4

RIGHT-LEFT ASYMMETRY OF TYROSINE HYDROXYLASE (TH) ACTIVITY IN RAT MEDIAN EMINENCE (ME): INFLUENCE OF BAROREFLEX NERVES. N.Alexander[1,*], N. Kaneda[2], A. Ishii[3], M. Mogi[3], M.Harada[3], T.Nagatsu[2]. [1]Dept. of Med.,Univ. of So. Calif., Los Angeles, CA 90033, [2]Dept. of Biochem., Nagoya Univ. Sch. of Med., Nagoya 466, Japan, [3]Dept. of Oral Biochem., Matumoto Dental Coll.,Shiojiri, Japan.

TH activity in hypothalamus is affected by the arterial baroreflex system (Alexander and Morris Am. J. Phys. 255: R768, 1989). We tested whether sinoaortic denervation (SAD) would differentially affect TH on left and right sides of the brain since the left aortic nerve is larger and of different origin than the right. Unexpectedly, intact control rats showed significantly less (50%) TH activity on the left than right side of the ME and arcuate (ARC) as determined by in vivo (NSD 1015 treatment) and in vitro methods of enzyme measurement in micropunched brain samples. No such TH asymmetry was found in paraventricular or caudate nuclei of the same rats. Unilateral and bilateral SAD enhanced the asymmetry of TH activity in ME and ARC. Further study revealed that immuno-specific TH protein was significantly reduced on the left sides of ME and ARC thereby accounting for the asymmetry of TH activity. The physiological significance of this specific right-left TH asymmetry in the hypothalamus remains to be determined.

540.5

TRADITIONAL PROPYLTHIOURACIL DOSES USED FOR INDUCING HYPOTHYROIDISM ARE TOO HIGH. T. T. Sherer* and R. J. Bull. Pharmacology/Toxicology Program, College of Pharmacy, Washington State University, Pullman, WA 99164.

Propylthiouracil (PTU) is commonly used in experimental models to induce a hypothyroid state. However, information on the minimum doses required to maximally suppress thyroid function of fetal and neonatal models is not available. To obtain appropriate dose-response information pregnant Sprague-Dawley rats were exposed to various levels of PTU (0, 50, 100 and 200 mg/L) in the drinking water from gestational day 20 to postnatal day 21. Litters were reduced to 8 pups on the day of birth (postnatal day 0) and blood was collected from 2 pups per litter on postnatal days 1, 6, 12 and 21. Plasma thyroid hormone levels (Amerlex RIA kit, NEN) were maximally reduced with all dose levels of PTU, compared to controls. Pups from all treated litters displayed classical signs of hypothyroidism. Cerebellums were collected from 2 pups per litter on postnatal days 1, 6, 12 and 21. Purkinje cell arborization observed using the Golgi-Kopsch method was reduced in treated pups at day 12 as has been reported with higher doses of PTU. These results indicate that hypothyroidism can be induced in neonates at doses at least 10-fold less than what has been commonly used in past studies. (Supported by NASA Grant No. NAG 9-226).

540.6

ANDROGEN CONTROL OF TYROSINE HYDROXYLASE AND NERVE GROWTH FACTOR IN THE PERIPHERAL SYMPATHETIC NERVOUS SYSTEM. M.E. Goldstein, A.W. Tank, L.H. Fossom*, M. Fahnestock and R.W. Hamill. Neurology and Pharm. Depts., Monroe Comm. Hosp./Univ. of Rochester, Rochester, N.Y. 14603, SRI Int., Mol. Biol. Dept., Menlo Park, C.A. 94025.

Previous studies have demonstrated that the sympathetic hypogastric ganglion (HG) is dependent upon the continued presence of testosterone for normal maintenance of tyrosine hydroxylase (TH) mRNA, protein, and activity. The present experiments show that androgen replacement therapy restores these levels in the HG of castrated rats. The reduction in TH mRNA was examined further by in situ hybridization using a riboprobe synthesized from pGEM-3 into which a fragment of TH cDNA has been inserted and immunocytochemistry with a TH-specific polyclonal antibody to determine whether specific subpopulations of neurons within the HG are responding to castration. The results support the finding that TH mRNA levels become reduced following castration and reveal that the reduction is restricted to a specific TH-positive population of cells in the HG. Finally, we have further investigated whether testosterone is acting directly on the TH gene, or acting indirectly by affecting nerve growth factor (NGF) levels in a major target of the HG, the vas deferens (VD). Dot blot analysis of NGF mRNA in the VD reveals a progressive decrease in NGF mRNA in the VD 1, 2, 4 and 8 weeks following castration and levels are restored following treatment with testosterone. It is possible that TH levels in the HG are dependent upon the continued presence of NGF in the VD which is in turn dependent upon the continued presence of testosterone.

540.7

FURTHER STUDIES ON LHRH NEURONAL RESPONSIVENESS TO NOREPINEPHRINE (NE). **C.A.Barraclough and R.D.Hartman.** Dept. of Physiol., Univ. Maryland, Sch. of Med., Baltimore, MD 21201.

Previously, we reported that LHRH neurons of estrogen-treated, ovariectomized rats are severely limited in their responsiveness to NE. In the present study, we examined the combined effects of medial preoptic area (MPOA) electrical stimulation (ES for 15 min) and ICV or MPOA NE infusions on LH release. Plasma LH increased during ES to reach peak values at 15 min and declined rapidly thereafter. ICV NE after ES (16 min) had no amplifying effect on LH release although the rate of decline was reduced. Blockade of inhibitory beta adrenoreceptors (propranolol) prior to MPOA-ES + ICV NE did not improve peak responses but resulted in a more sustained elevated release of LH. Direct MPOA infusions of NE as single or multiple pulses produced only modest increases in plasma LH. Single NE infusions 5 or 16 min after beginning ES had no amplifying effect on LH release but when dual NE infusions were made, plasma LH secretion was sustained at peak levels for an additional 30 min. Neither propranolol nor pargyline (MAO inhibitor) improved the responses obtained after MPOA NE infusions with or without combined ES. Thus, LHRH neuronal responses to NE differ considerably after MPOA-ES versus electrochemical stimulation (ECS). ECS produces an irritative lesion which activates LHRH neurons for a prolonged period (120 min), whereas, ES only is effective for the duration of the stimulus (15 min). Consequently, LH peak values achieved after ECS + NE are equivalent to those which occur during the spontaneous LH surge. In contrast, when NE is introduced after terminating ES, it is unable to further activate LHRH neurons as they rapidly reacquire their unresponsive characteristics. Seemingly, in the normal sequence of events, a critical endogenous inhibitory control first must be removed before NE can trigger a preovulatory LH surge.

540.8

EFFECTS OF ORCHIDECTOMY ON BASAL AND STRESS-INDUCED DECREASES IN TUBEROINFUNDIBULAR DOPAMINERGIC (TIDA) NEURONAL ACTIVITY. T.W. Toney*, K.J. Lookingland and K.E. Moore (SPON: J.L. Bennett). Department of Pharmacology and Toxicology, Michigan State University, East Lansing, MI 48824.

The basal level of activity of TIDA neurons is higher in female than in male rats. Furthermore, restraint stress increases plasma levels of prolactin in both male and female rats, but only in the female is there an accompanying stress-induced decrease in TIDA neuronal activity. In the present study TIDA neuronal activity was estimated by measuring in the median eminence: 1) 3,4-dihydroxyphenylacetic acid (DOPAC) concentrations and 2) the accumulation of 3,4-dihydroxyphenylalanine (DOPA) following the administration of a decarboxylase inhibitor. One and two weeks following orchidectomy DOPAC concentrations and DOPA accumulation in the median eminence were increased when compared with intact control animals. Following 30 min of restraint both DOPAC concentrations and DOPA accumulation were decreased in orchidectomized but not in intact male rats. It is concluded that circulating androgens inhibit TIDA neurons and play a role in the lack of response of these neurons in intact male rats to restraint stress. (Supported by ADAMHA grant MH42802.)

540.9

CENTRAL SOMATOSTATIN/GRF INTERACTIONS: MORPHOLOGIC AND PHYSIOLOGIC EVIDENCE. G.F. McCarthy*, A. Beaudet, and G.S. Tannenbaum. Departments of Pediatrics, Neurology and Neurosurgery, McGill University, Montreal, Quebec H3H 1P3.

Several lines of evidence suggest that somatostatin (SRIF) may directly interact with GH-releasing factor (GRF)-containing neurons centrally to modulate GH secretion. To determine whether such interactions are implicated in regulating GRF content of arcuate (ARC) neurons, and consequently GH secretion, we examined the effects of the potent SRIF-depleting agent, cysteamine (CSH), on ARC GRF immunoreactivity and on pulsatile GH secretion. Serial sections of hypothalami of CSH-treated rats studied with PAP immunocytochemistry demonstrated a striking increase in both the number (126%) and staining intensity of GRF-immunoreactive cells compared to controls. This increase was significant ($P<0.01$) at all levels of the ARC nucleus but most pronounced within the caudal tier. Administration of CSH (90 mg/kg iv) to free-moving rats caused a marked elevation of basal plasma GH levels (8.2 ± 1.2 vs. 1.8 ± 0.4 mg/ml; $P<0.01$) which was prevented by passive immunization with a specific antiserum to GRF (2.8 ± 0.8 vs. 9.3 ± 2.0 ng/ml in normal serum + CSH-injected controls; $P<0.02$) indicating a role for endogenous GRF in mediating this response. Taken together, these findings support the concept that SRIF exerts a tonic inhibitory influence on GRF-containing ARC neurons which may be an important mechanism in the physiological control of pulsatile GH secretion.

540.10

ADRENERGIC INNERVATION OF SOMATOSTATIN /SS/ AND GROWTH HORMONE-RELEASING HORMONE (GH-RH) SYNTHESIZING HYPOTHALAMIC NEURONS IN THE RAT. Zs. Liposits*, E. Hrabovszky* and W.K. Paull*. (SPON: J. Dexter). Depts. of Anatomy, Univ. Med. Sch. Pécs, Pécs, Hungary, 7643 and Univ. of Missouri-Columbia, Columbia, MO, U.S.A, 65212.

In order to determine whether adrenergic, phenylethanolamine-N-methyltransferase (PNMT)--immunoreactive (IR) axons terminate on hypophysiotrophic SS-IR and GH-RH-IR neurons, respectively, immunocytochemical double labelling studies (Liposits et al. Histochemistry 85:95, 1986) were performed in the hypothalamic paraventricular (PVN) and arcuate (ARC) nuclei of the rat. Both SS-IR neurons residing in the periventricular part of the PVN and GH-RH-IR cells located in the ARC received PNMT-IR axons on their cell bodies and dendrites. The ultrastructural analysis of the juxtaposed elements revealed axo-somatic and axo-dendritic synapse formation of PNMT-IR axons with SS- and GH-RH-IR neurons. These studies demonstrate that PNMT-IR afferents innervate both hypophysiotrophic SS- and GH-RH producing neurons and indicate the participation of the adrenergic system in the central regulation of growth horhormone secretion. Supported by grants from NIH (NS 19266), NSF (INT 8703030) and MTA (OTKA 104).

540.11

HUMAN GROWTH HORMONE RESPONSE TO OVINE CORTICOTROPIN RELEASING FACTOR. J.C. Ritchie, R.L. Scotch*, J.T. Walker*, K.R.R. Krishnan*, D.A. Reed*, C.B. Nemeroff. Depts. of Psychiatry and Pharmacology, Duke Univ. Med. Ctr., Durham NC 27710.

We present here the results of our study on the effect of ovine CRF on growth hormone secretion in normal human volunteers and patients with DSM IIIR major depression. All subjects gave informed consent prior to the initiation of the study. The CRF challenge was performed in standardized fashion. Blood samples were obtained at -15, 0, +15, +30, +45, +60, +90, +120, +150, +180, and +210 minutes. Samples were analyzed for cortisol, ACTH, and growth hormone. The growth hormone responses were much more variable and delayed compared to those of cortisol and ACTH. 5 of 15 control subjects and 2 of 17 depressed patients had no response to the infused ovine CRF (i.e. no increase over baseline). The average peak response for those controls who did respond was 5.11 ng/mL and this peak occurred at 180 minutes after the CRF bolus. The average peak response for responders in the depressed group was 3.89 ng/mL and occurred 120 minutes after the CRF bolus. These results support the observation that the pituitary of depressed patients is dysregulated compared to normals.

Supported by NIMH grants MH 42088 and MH 39593

540.12

ACTIVATION OF BASAL HYPOTHALAMIC MU, NOT DELTA OR KAPPA, -OPIOID RECEPTORS STIMULATES GROWTH HORMONE SECRETION IN THE UNRESTRAINED RAT. J.O.Willoughby and R. Kapoor*. Centre for Neuroscience and Department of Medicine, Flinders University of South Australia, Bedford Park, South Australia, 5042.

Growth hormone (GH) is released by systemically administered opioids and several opioid receptor subtypes have been implicated. We prepared anesthetised male albino rats with permanent indwelling intracerebral guide cannulas and a venous catheter and after recovery microinjected mu, delta and kappa opioid agonists into the medial basal hypothalamus, adjacent to GH releasing factor (GRF) neurons. GH concentrations were measured in 15 minute blood samples taken before and after injections which were in 0.25 ul Ringer. Average GH levels were increased by injection of the mu agonist D-Ala,Gly-ol Enkephalin (DAGO) at a minimum effective dose of 0.0001 nanomole. The minimum effective dose of the delta agonist D-Pen,D-Pen Enkephalin (DPDPE) and kappa agonist U50,488-H was 1.0 nanomole. GH secretion caused by DAGO was blocked by systemic pretreatment with naloxone 5 mg/kg. We conclude that mu opioid receptor activation on or close to GRF neurons causes GH secretion. The effectiveness of 10,000-fold higher doses of DPDPE and U50,488-H in stimulating GH suggests that they may be non-specifically activating mu receptors. Supported by the National Health and Medical Research Council and the Neurosurgical Research Foundation of South Australia.

540.13

NEUROPEPTIDE Y (NPY) CONTROLS LUTEINIZING HORMONE (LH), PROLACTIN (PRL) AND GROWTH HORMONE (GH) IN RATS. V. Rettori*, L. Milenkovic*, M. Riedel* and S.M. McCann (SPON: G. Moushegian). Dept. Physiology, Neuropeptide Div., Univ. Texas Southwestern Med. Ctr., Dallas, Texas 75235.

Earlier studies in ovariectomized and intact male rats have shown that intraventricular injection of neuropeptide Y inhibits release of GH and LH without producing significant modification of plasma FSH and TSH. In the male low doses of NPY elevate prolactin. To assess the physiologic significance of these actions, we injected a highly specific anti-NPY serum (aNPY) into the third cerebral ventricle (3V) of unrestrained male rats and measured plasma GH, PRL, LH and TSH by blood sampling via indwelling jugular catheters. 3V-injection of aNPY (2 µl of 1:10 dilution) caused a significant elevation of plasma GH levels after 3 and 4 hrs compared to the values in NRS (1:10)-injected rats. To determine if these changes were due to alterations in pituitary responsiveness to somatostatin, the rats were injected i.v. with a challenge dose of somatostatin (100 µg) 2 hrs after previous injection of aNPY or NRS, and blood samples were taken every 10 min for 30 min. The responses did not differ in both groups. 3V-injection of aNPY reduced plasma PRL levels after 180 min and elevated plasma LH levels significantly after 120 min without altering plasma TSH and FSH levels. Since the effects of the antiserum injection were opposite to those of the peptide itself, the results support the physiologic significance of neuropeptide Y in the control of GH, LH and prolactin by actions either directly on the pituitary and/or on the hypothalamus. Supported by grant HD09988.

540.14

Cysteamine-Induced Changes in Galanin in the Rat Brain and Pituitary. C.M. Milbury, J.B. Martin and J.I. Koenig, Neurology Service, Massachusetts General Hospital and Harvard Medical School, Boston, MA 02114.

Cysteamine has previously been shown to reduce hypothalamic somatostatin concentrations and interrupt growth hormone(GH) secretory rhythms. However, the actions of cysteamine appeared to be restricted to somatostatinergic neurons. In an effort to determine a physiological role for galanin(GAL) in the regulation of GH secretion, we have studied the effect of cysteamine treatment on brain and pituitary stores of GAL. Male rats were treated with cysteamine(300 mg/kg) 4 and 24 hrs before sacrifice. GAL concentrations were determined in 2N acetic acid extracts of brain and pituitary using a previously characterized RIA. The concentration of GAL in the median eminence was reduced 37.5% from 323±56 ng/mg protein 24hrs after cysteamine treatment. However, cysteamine lowered GAL concentrations by 50% in the posterior pituitary 4 hrs but not 24 hrs after treatment (control values were 39.8±4.5 ng/mg protein). Anterior pituitary stores of GAL transiently increased after cysteamine treatment from 0.7±0.2 ng/mg protein to 1.4±0.5 ng/mg protein. No changes were observed in the hypothalamic fragments analyzed. These studies suggest that cysteamine, a factor known to alter GH secretion, produces marked changes in GAL and are consistent with a stimulatory role for GAL in the regulation of GH secretion.

540.15

Presence of Galanin-like Immunoreactive Material in the Hypophysial Portal Blood of the Rat. J.I. Koenig and S.M. Gabriel. Neurology Service, Massachusetts General Hospital and Harvard Medical School, Boston, MA 02114 and Department of Psychiatry, Mount Sinai School of Medicine, New York, New York 10029.

Recent studies have demonstrated that galanin (GAL) may play a modulatory role in episodic growth hormone(GH) secretion in the rat. GAL appears to act in the hypothalamus and pituitary to stimulate GH secretion. Furthermore, GAL is found in high concentrations in the median eminence in close proximity to the hypophysial portal capillary loops. These studies suggest that GAL may be secreted into the portal vasculature. In the present study, we have sought to determine if GAL is present in the rat portal circulation. Adult male rats were anesthetized with urethane or pentobarbital. Hypophysial portal blood was collected as previously described (Endocrinology 118:2534). The concentration of GAL in the portal plasma was determined using an antibody developed against rat GAL conjugated to KLH with carbodiimide. The concentration of GAL in the hypophysial portal blood was 82.1±9.2 pg/ml. In the same animals systemic concentrations of GAL were 20.9±2.4 pg/ml. These data suggest that GAL may be secreted from the hypothalamus and be carried to the pituitary gland where it may augment the stimulatory effects of the hypothalamic factors regulating GH secretion.

540.16

BODY WEIGHT AND SWIMMING BEHAVIOR IN RATS WITH SUBDURAL CAPSAICIN. I. Zarco de Coronado, M. A. Paredes Cuanalo*, M. Aguilar Díaz*, and I. A. Coronado Zarco*. Departamento de Fisiología, Facultad de Medicina. Universidad Nacional Autónoma de México A.P. 70250.

It has been reported that intraperitoneal capsaicin in new born rats determines an increase in water and food ingestion, a slower swimming behavior evolution and a bigger hypophysis. This study has the proposal to know whether or not these effects are centrally mediated. Three groups of 5 days old rats were used. One group received the subdural administration of 0.5 uL of 20% Capsicum annum extract. The control groups was subdurally injected with the vehicle. The third group did not received subdural inyection. The body weight of treated and control animals was inferior from the normal animals. A delay of 2 days was observed in the swimming behavior maduration of the control and treated animals.

These data support the hypothesis that both, peripheral and central SP pathways participate in somatovisceral activity integration, but the central capsaicin effects were masked by collateral actions of subdural injection.

540.17

STIMULATION OF INOSITOL PHOSPHOLIPID HYDROLYSIS BY EXCITATORY AMINO ACIDS IN RAT HYPOTHALAMUS: DEVELOPMENTAL PROFILE AND PHARMACOLOGICAL CHARACTERIZATION. M.A. Sortino, F. Nicoletti* and P.L. Canonico. Department of Pharmacology, University of Catania, Italy.

Excitatory amino acids stimulate inositol phospholipid hydrolysis in slices prepared from rat hypothalamus. As occurs in other brain regions, this stimulation is much greater during the first 15 days of postnatal life (up to 15 fold increase with quisqualate or ibotenate), progressively declines during maturation, is still present after 30 days but disappears in slices from 2 month old animals. In hypothalamic slices, quisqualate is by far the most potent activator of inositol phospholipid hydrolysis (EC_{50} value = 2-5 uM), followed by ibotenate and glutamate, whereas N-methyl-D-aspartate (NMDA), kainate and aminohydroxymethylisoxazolepropionate (AMPA) are inactive. In hypothalamic slices from newborn animals, maximal stimulation of [^{3}H]-inositol-monophosphate formation by quisqualate, ibotenate and glutamate is greater than that induced by norepinephrine or carbamylcholine. Hypothalamic slices are a suitable model to study whether excitatory amino acid receptors regulate the pattern of neurohormone secretion during maturation.

540.18

PRENATAL vs. POSTNATAL EXPOSURE TO THE ACIDIC AMINO ACIDS GLUTAMATE AND ASPARTATE IN MALE AND FEMALE RATS. J. F. Rodriguez-Sierra and R. Earle. Depts. Anatomy and Pathology, Univ. Nebraska Med. Ctr., Omaha, NE 68105.

The arcuate nucleus of the hypothalamus (ARC) is known to be a site of action for the excitoxic acidic amino acids glutamate (Glu) and aspartate (Asp). The toxicity is expressed during the neonatal life of the rat. Authors claim neurotoxic effects in the medulla, particularly on the innervation of the vascular bed of the area postrema, but data about ARC neurotoxicity during fetal exposure is not available. This study was performed to compare prenatal to postanatal exposure. In addition, we measured the concentration of Glu and Asp in fetal and maternal circulation after Glu exposure. Sprague-Dawley rats were injected with Glu or Asp (monosodium salts 4 mg/g bw, sc) on days 1, 3, 5, 7, and 9. Animals were sacrificed and endocrine glands and body weights were recorded. Brains were examined for lesions in the ARC. Another group of rats were exposed to Glu or Asp during pregnancy (during the last week of pregnancy). Animals were allowed to go to term and the young sacrificed at different intervals for determination of ARC lesions. Other pregnant animals were sacrificed at different intervals after one injection of Glu, the blood of mothers and of fetuses collected and the plasma stored frozen until analysis. Plasma glutamate levels were determined by amino acid analysis, using DC-6 cation exchange resin, lithium citrate buffers, and a post-column ninhydrin reactor. Neonatal treatment with Glu or Asp resulted in typical ARC lesions that were accompanied by endocrinopathies. Prenatal treatment did not result in endocrinopathies nor did the animals showed signs of ARC lesions. The levels of Glu increased significantly in the blood of fetuses exposed to Glu treatment. The results suggest that lack of neurotoxic effects of Glu or Asp might be due to the absence of a functional receptor system in the ARC for the acidic amino acids and soon after birth the receptor system becomes functional. (This work was partially supported by the NIH (HD-13219)

540.19

Neurotoxicity of Kainic Acid to Luteinizing Hormone-Releasing Hormone Neurons: Evidence from In Vitro Secretion. L.L. Lanker* and R.W. Clough. (SPON: B. Yau) Dept. of Anatomy, Southern Illinois Univ. Sch. of Med., Carbondale, IL 62901

We have previously demonstrated that stereotaxic infusion of kainic acid (KA) into the medial preoptic or the medial septal areas effectively blocks estrogen and estrogen + progesterone stimulated luteinizing hormone secretion (an E + P induced LH surge) in addition to causing pubertal delay (Clough, et al., Neuroendocrinology 33:363, 1981; Biol. Reprod. 35:1269, 1986). It was presumed that these effects were a result of the excitotoxic effect of KA on neurons in the infusion area, including LHRH neurons. The present study has used a perifusion culture system to determine the effects on KA on LHRH release from organotypic preoptic-mediobasal hypothalamic (POA-MBH) explants. POA-MBH explants, were removed from female rats and were placed individually into 200 ul chambers housed in a gas and heat exchanger. Each explant was thus perifused with warmed and oxygenated Gibco F-12 media at a rate of 25 ul/min. After equilibration, explants were exposed to culture medium containing 1 ug/ul KA. LHRH release over time was assessed by radioimmunoassay. Infusion of KA caused an acute increase in LHRH release into the perifusion media followed by a gradual decline to sub-baseline levels. Exposure of explants to potassium chloride, subsequent to KA, resulted in no change in LHRH release. In other studies, we have demonstrated KCl responsiveness of POA-MBH explants in LHRH release for several days in culture. Although these studies demonstrate that LHRH neurons are disrupted by the excitotoxin KA and that lack of E + P responsiveness following intrathecal infusion (cited above) may be a result of LHRH neuron destruction, this study does not suggest that E + P act directly on LHRH neurons to induce an LH surge. Rather, this study supports the LHRH neuron as the final common pathway to the adenohypophyseal gonadotrophs. Supported by HD 24426 (RWC).

540.20

IMMUNOHISTOCHEMICAL LOCALIZATION OF BASIC FIBROBLAST GROWTH FACTOR (bFGF) IN RAT HYPOTHALAMUS. P.N. Riskind, P. J. Apostolides*, M. DiFiglia, C.G. Caday*, M. Klagsbrun* and S.P. Finklestein. Dept. of Neurology, Mass. General Hospital, Dept. of Surgery, Children's Hospital Medical Center, and Harvard Medical School, Boston MA 02114.

Immunohistochemical analysis of hypothalamic bFGF was performed on tissues from six mature male rats using three specific polyclonal rabbit antisera directed against bFGF. Intense bFGF-staining was noted within axon terminals in the median eminence, especially in the internal lamina, and in the organum vasculosum lamina terminalis (OVLT). Strong bFGF-staining was detected in axon terminals in the subfornical organ (SFO), periventricular nuclei (Pe) and arcuate nuclei (AN), and in fibers in the Pe. Strong cell-labeling was present in neurons within the supraoptic nuclei (SON), in the magno- and parvo-cellular aspects of the paraventricular nuclei (PVN) and in the Pe. These results suggest a physiologic role of bFGF in hypothalamic structures subserving control of neuroendocrine function. Supported by NIH 26252, NIA AG 08207 and AHA 88725.

541.1

TEMPORAL AND SPATIAL DISTRIBUTION OF A *DROSOPHILA* PROTEIN WHICH RESEMBLES BETA-AMYLOID PRECURSOR. L.Luo, L.Martin-Morris, and K.White. (SPON: John Lisman) Dept. Biology, Brandeis Univ., Waltham, MA 02254

We have identified a transcript in *Drosophila* which encodes a putative membrane-spanning polypeptide similar to Alzheimer disease associated ß amyloid protein precursor (Rosen *et.al. PNAS* 86:2478, 1989; Kang,*et.al. Nature* 325:733, 1987). This transcript and the *Drosophila* vital locus *ventral nervous system condensation defective (vnd)* map within the same genomic interval. Mutations in the *vnd* locus cause morphological defects in the CNS and result in embryonic lethality.

Polyclonal antibodies were generated using bacterial fusion protein system. The affinity purified antibodies recognize two bands of molecular weight 130 and 145 Kd in *Drosophila* embryo extracts. They are first detected in 9 to 12 hour extracts and are present throughout embryogenesis. By immunocytochemistry this antigen is present predominantly in the nervous system, throughout the CNS and PNS. The antigen is also present in larva CNS and in the developing photoreceptor cells. Studies of the later stages and *vnd* mutants are in progress.

Based on the spatial, temporal distribution, and molecular structures predicted from the sequence, we propose that this *Drosophila* molecule might play some function in neuronal cell-cell communication in the development and maintenance of the nervous system.

541.2

A FRAGMENT OF THE ALZHEIMER AMYLOID PRECURSOR IS TOXIC TO DIFFERENTIATED NEUROBLASTOMA CELLS. I. Slavc*, L.R. Dawes, R.L. Neve, B. Korf*. The Children's Hospital, Boston, MA 02115 (SPON: R. Jacobson)

Stable PC12 transfectants expressing the carboxyterminal 105 amino acids (AB1) of the Alzheimer amyloid precursor protein (APP) have been shown to degenerate during nerve growth factor (NGF)-induced differentiation into neuronal cells. Conditioned medium (CM) from these cells, and from NIH 3T3 cells transfected with the same APP fragment, is also toxic to neurons in primary rat hippocampal cultures. We tested the generality of this phenomenon by determining the effect of AB1 CM from 3T3 cell transfectants on the human neuroblastoma cell line LAN-5 which had been induced to differentiate with $2x10^{-6}$M retinoic acid (RA). AB1 CM was added to LAN-5 cells after 4 d in culture with RA. 50-75% of the RA-treated cells died after 5-6 d in AB1 CM. In contrast, LAN-5 cells in CM from nontransfected 3T3 cells differentiated normally in response to RA. The degenerative effect of the AB1 CM depended on the degree of differentiation achieved by LAN-5 cells before the addition of AB1 medium. Our data suggest that the neurotoxic activity of the APP AB1 fragment on rat PC12 cells induced to differentiate with NGF and on primary neuronal cultures can be reproduced in a human neuroblastoma line induced to differentiate with RA. Thus, the neuronal degeneration caused by AB1 is specific to the neuronal phenotype and is not dependent on NGF.

541.3

IMMUNOCYTOCHEMICAL STUDIES OF BRAIN AND PC12 CELLS USING ANTIBODIES TO THE AMYLOID PRECURSOR PROTEIN C.A. Marotta, E.C. Walcott, B.Tate-Ostroff and R.E. Majocha (SPON: S. Matthysse) Mass Gen Hosp, Harvard Med Sch, Boston, MA; McLean Hosp, Belmont, MA

We developed antibodies to APP domains for immunostaining of human brain and PC12 cells. Antibodies are to the N terminal region (N1), the extracytoplasmic domain (P2; Tate-Ostroff et al., PNAS 86, 745; 1989), the B-peptide region (A4; Majocha et al., PNAS 85, 6182; 1988), and the C terminal region (C1). Brain and PC12 cells stained with N1, P2 and C1 antibodies. Alzheimer brain showed N1, P2 and A4 staining associated with thioflavin positive plaques. Conditioned media from normal PC12 cells was found to inhibit the staining of brain tissue by all APP antibodies except A4 and C1, indicating that PC12 cells may shed only the extracytoplasmic domains of the APP. These studies provide insight into the location of APP domains in PC12, Alzheimer and control brains, as well as the processing of the molecule. Supported by AG02126, AHAF and the Sandoz Foundation.

541.4

REGULATION AND RELEASE OF ALZHEIMER AMYLOID PRECURSOR ROTEIN IN MEMBRANE DAMAGED NEURONAL CULTURES, F. Baskin*, R.N. Rosenberg, and S.A. Stein, Dept. of Neurol., Univ. Texas Southwestern Medical Center, Dallas, Texas 75235.

Extracellular amyloid is seen in specific areas of early Alzheimer's Disease (AD) brain. The APP gene, encoding the precursor of the A4 amyloidic peptide, is expressed as APP_{695} and/or $APP_{751/770}$, the latter containing a protease inhibitor sequence. A4 has been described as a neurotoxic or neurotrophic marker of AD altered APP degradation. We are using clonal neuronal cultures to examine the regulation of the APP gene and the importance of prior membrane damage, APP form and AD or control proteases.

Our PC-12 and immortalized olfactory bulb clonal cells express only $APP_{751/770}$ whereas IMR-32 cells equally express APP_{695} and $APP_{751/770}$ mRNAs. Consistent with their effects on protein kinase C (reduced in AD brain and fibroblasts), NGF and phorbol ester increase APP mRNA whereas H7 decreases it. We are investigating the effect of interleukin-1, increased in injured brain, in these cultures.

Analyses of ^{125}I-proteins released from cultures grown with 0.005% SDS and other membrane damaging agents indicate the increased release of a 60kd peptide specifically binding A4 elicited antiserum. This suggests the importance of prior membrane damage in AD amyloidogenesis. We are examining the differential degradation of APP-like proteins by fractionated AD proteases and "amyloid enhancing factor".

541.5

SECRETION OF AMYLOID β-PROTEIN PRECURSOR. G.M. Cole, T. Huynh*, D. Schubert*, and T. Saitoh. (SPON: W.C. Wiederholt). University of California, San Diego, Dept. of Neurosciences, M-024, La Jolla, CA 92093, Salk Institute for Biological Studies, La Jolla, CA 92138, U.S.A.

We reported part or all of amyloid β-protein precursor (ABPP) is released into the conditioned medium (CM) of PC12 and other cells (Schubert et al., Science 241:223- 226, 1988; Schubert et al., PNAS 86:2066-2069, 1989; Uéda et al., Ann Neurol 25:246-251, 1989). Both the insert and non-insert immunoreactive forms of ABPP are secreted, but C-terminal antiserum reacts only with the membrane form. The size of the secreted ABPP is similar to the size of the full length membrane form which suggests ABPP is normally cut in or near the amyloidogenic β/A4 region which is next to the membrane. Here, we show the majority of ABPP is in the CM of human neuroblastoma, teratocarcinoma, medulloblastoma and retinoblastoma and glioma cells demonstrating that ABPP is generally secreted. Treatment of cells with leupeptin, chloroquine or ammonium chloride dramatically increases the soluble intracellular and membrane forms of ABPP suggesting a possible lysosomal pathway in ABPP metabolism. With lysosomal protease inhibitors similar increases are also seen in a small C terminal immunoreactive membrane fragment suggesting it may also undergo lysosomal processing. Experiments are underway to determine the relationship between normal secretion and lysosomal processing.

541.6

ROLES FOR INTERLEUKIN-1 AND NERVE GROWTH FACTOR IN AMYLOID FORMATION IN ALZHEIMERS'S DISEASE ? F. Berkenbosch*, D. Casper, R. Hellendall*, V. Friedrich Jr.*, L. Refolo*, D. Lahiri*, M. Blum, and N. Robakis. Fishberg Res. Center in Neurobiology, Dept. Psychiatry and Molecular Biology, Mount Sinai School of Medicine, New York, NY 10029.

Nerve growth factor (NGF) can induce secretion and can alter processing of beta-amyloid precursor protein (APP) in several cell lines (Refolo et al., submitted). The monokine interleukin-1 (IL-1) in turn can regulate NGF production (lindholm et al., Nature 330,658 (1987)). Moreover, IL-1 also can regulate APP mRNA levels, as can be concluded from our study showing increased APP mRNA levels in human-fibroblasts in response to IL-1. These studies suggest the existence of an IL-1-NGF cascade regulating synthesis and maturation of APP. In order to investigate whether such cascade operates in CNS tissue, we cultured hippocampus and frontal cortex tissue from the rat E18 fetus. Interestingly, in the same areas, we found by in situ hybridization analysis using a 30-mer IL-1 beta oligonucleotide abundant cells expressing IL-1 mRNA in the adult rat. Since hippocampal and cortical cultures contain neurons and glia, we also established cultures of glia alone. All cultures were characterized by immunostaining using antibodies to neurofilament (neurons), glial fibrillary acidic protein (astrocytes), galactocerebroside (oligendrocytes), O4 and A2B5 (glial precursors). In the hippocampal cultures, IL-1 increased mRNA levels coding for NGF. Western blot analysis with different antibodies to APP showed the presence of APP in hippocampal and glial cultures. In neurons the APP staining was distributed over the cell bodies and neurites. In glial cells, APP staining was present in astrocytes type I and appeared to be associated with the cytoskeleton of the cells. Experiments to determine whether an IL-1-NGF cascade controls APP production and processing are in progress.

541.7

TRANSFECTION OF B-AMYLOID INTO CHOLINERGIC NEURONAL CELL LINES. A. Dagenais*, N.R. Cashman, J.P. Julien*, D. Gauvreau* and J. Nalbantoglu*. (SPON: S. Gauthier). INRS-Santé, Pointe-Claire, Qué., Canada, H9R 1G6; Institut du Cancer de Montréal, Montréal, Qué. Canada, H2L 4M1 and Montreal Neurological Institut, Montréal, Qué. Canada, H3A 2B4.

The β-amyloid protein (A4) is found in neuritic plaques of Alzheimer and Down Syndrome patients. This protein is part of a high molecular weight membrane-bound precursor expressed in the brain and a wide variety of tissues. The localization of the amyloid gene to chromosome 21 suggests that an overexpression of the protein might be responsible for the deposition of amyloid in the plaques. To verify this hypothesis, we have transfected a plasmid containing the C-terminal domain of the amyloid precursor, spanning from the A4 region to the cytoplasmic carboxy-end; the recipient cell lines, PC-12 and NG-108, can be differentiated in vitro into cholinergic neurons in the presence of NGF and dibutyryl cAMP respectively. The amyloid coding sequences are linked to the neurofilament NF-L promoter to allow a high level of expression of the amyloid transcript. We have isolated several clones in which this construct is integrated to genomic DNA. We are in the process of characterising these transfectants and of studying the effect of β-amyloid overexpression on the viability and differentiation of these neuronal cell lines. Supported by MRC Canada grant MA-10407.

541.8

ANTIBODIES TO SYNTHETIC PEPTIDE SEQUENCES OF THE BETA AMYLOID PROTEIN PRECURSOR: DISTRIBUTION IN THE RAT CNS. J.G. Beeson[1], E.R. Shelton[2], J.J. Nestor, Jr.[2], H.W. Chan[2], and F.H. Gage[1]. (SPON: R. Whiting) Dept. of Neurosciences, UCSD, La Jolla, CA 92093, and Institute of Bio-Organic Chemistry, Syntex Research, Palo Alto, CA.

In our studies to date, we have used a battery of antibodies raised against different synthetic peptide sequences of human APP in the immunohistochemical staining of young adult rat brain.

Amongst the antibodies used are: 1) 679, anti C-terminus (raised against amino acids 679-692 of APP695): 2) 612, anti-amyloid (amino acids 612-624); 3) j284, raised against the junctional region of APP 695 (amino acids 284-299); and 4) i291 and i324, raised against different regions of the APP751 insert (amino acids 291-305 and 324-336 respectively).

The application of these antibodies resulted in specific staining which in some cases differed from mRNA studies. Pretreatment of tissue with protease k was required to produce significant staining with i291 and 612, while the use of protease k pretreated glutaraldehyde fixed tissue resulted in striking cellular staining with i324.

We will present data on the distribution of the antibodies in aged and young rats. Further we are investigating the role of NGF in the regulation of subspecies of APP.

541.9

AMYLOID BETA PROTEIN PRECURSOR IN CEREBROSPINAL FLUID IN ALZHEIMER'S DISEASE. D. Galasko,* G. Cole,* L.J. Thal,*+ T. Saitoh.* *Dept. Neurosci., UCSD, La Jolla. CA 92093; +Neurology Ser., VA Med. Ctr., San Diego, CA 92161

Beta protein, a 4.2 kD polypeptide, is a major component of amyloid deposits in Alzheimer's disease (AD). Three mRNAs code for amyloid beta protein precursor (ABPP) molecules of 695, 751 and 770 amino acids. ABPP is proposed to be an integral membrane protein with a small C-terminal cytoplasmic domain, a transmembrane domain containing part of the beta protein sequence, and a large extracellular N-terminal domain, which contains an insert resembling a protease inhibitor in the 751 and 770 amino acid forms. To obtain an index of ABPP metabolism in the brain, we measured ABPP immunoreactivity in human cerebrospinal fluid (CSF), by immunostaining Western blots. A polyclonal antibody to an amino acid sequence in the N-terminal region of ABPP stained bands of approximate Mr 88 and 100 kD. These two bands corresponded to similar bands in blots of conditioned medium from cultured PC12 cells and the soluble fraction of AD brain homogenates. An antibody to a C-terminal sequence failed to stain high molecular weight bands in CSF, but stained at least two bands of Mr 110-120 in brain homogenates. This suggests loss of the C-terminal portion when ABPP is secreted into CSF. The CSF bands appear to stain more intensely on average in AD than in controls. CSF ABPP may be a biological marker of amyloid deposition in AD.

541.10

VASCULAR BASEMENT MEMBRANE COMPONENTS ASSOCIATED WITH SENILE PLAQUE AMYLOID. L.S.Perlmutter, D.Saperia, J.Wu*and H.C.Chui* Univ.Southern California Sch.Med., Dept.Neurol., RMR 407, Los Angeles, CA 90033

Microangiopathy (pathologic alterations of the capillary bed) may be a concomitant of Alzheimer's disease (AD): capillary fragmentation is correlated with neuronal loss (Zarow et al, this volume), vascular basement membrane (VBM) is thickened and vacuolized (Perlmutter et al, Soc. Neurosci.Abstr., 1988, 14, 639), and one constituent of VBM, heparan sulfate proteoglycan (HSPG), has been associated with amyloid of senile plaques (Perlmutter et al, submitted; Snow et al, Am.J.Path., 1988, 133, 456). Two additional intrinsic constituents of VBM, collagen type IV (CIV) and laminin, were examined in the present study. Immunocytochemically-labelled autopsy tissue was double-stained with a fluorescent marker for amyloid (thioflavine S). CIV-immunoreactivity (CIV-I) outlined small blood vessels and the capillary bed. Punctate accumulations of CIV-I were often associated with amyloid cores. Small blood vessels, a portion of the capillary bed, and a subset of amyloid cores were laminin-immunoreactive (laminin-I). Ultrastructurally, CIV-I and laminin-I outlined the VBM; reaction product also decorated extravascular amyloid fibrils. The colocalization of several VBM constituents with the senile plaque suggests that the VBM may be a primary instigator in the pathogenesis of this lesion. (French Fdn & AG-07127 (LSP), AG-05142, AG-07624 (HCC,LSP))

541.11

CORRELATION BETWEEN MICROANGIOPATHY AND NEURONAL LOSS IN ALZHEIMER'S DISEASE. C. Zarow*[1], H.C. Chui*[2], E. Hsu*[2], and L.S. Perlmutter[2] (SPON: D. Saperia). Univ Southern Calif, [1]Ethel Percy Andrus Gerontol Ctr, Los Angeles, CA 90089; [2]Sch Med, Dept Neurol, Los Angeles, CA 90033.

Pathologic changes in the microvasculature (microangiopathy) accompany Alzheimer's disease (AD). Immunostaining for heparan sulfate proteoglycan (HSPG, vascular basement membrane component) reveals capillaries in non-AD patients orient from pia to white matter as an organized, contiguous, anastomosing structure. In AD patients, the microvasculature has lost this radial orientation, and appears broken into small, disconnected pieces. The relationship between degree of capillary disruption and neuronal loss, senile plaques, and neurofibrillay tangles, were examined. Number and area of HSPG-immunolabeled capillary fragments were determined with a Quantimet 970 image analysis system in the upper cortical laminae of temporal lobe. Densities of neurons, plaques and tangles were determined (camera lucida drawings of cresyl violet, Bielshowsky's silver stain sections). The numbers of 'small' (disrupted;<1000 um^2) or 'large' (contiguous;>1000um^2) immunostained capillary fragments were correlated with densities of neurons, plaques and tangles. 'Small' was inversely correlated with neuronal density ($r=-0.72$, $p<0.005$); no other significant correlations were found. This suggests that microangiopathy is common in AD and may provide another index of pathologic severity. (AG-05142; AG-07624 (HCC,LSP); French Fdn, AG-07127 (LSP))

541.12

MOLECULAR DISSECTION AND FUNCTIONAL ANALYSIS OF THE AMYLOID β-PROTEIN PRECURSOR. J.-M. Roch, M. Sundsmo*, P. Ward*, D. Schenk*, L. Refolo*, N. Robakis, and T. Saitoh. University of California, San Diego, School of Medicine, Dept. of Neurosciences, M-024, La Jolla, CA 92093, U.S.A., Athena Neurosciences, Inc., 800 F Gateway Blvd, South San Francisco, CA 94080, and Mt. Sinai School of Medicine, One Gustave L. Levy Place, New York, NY 10029, U.S.A.

Amyloid β-protein precursor (ABPP) was shown to be a growth factor-like molecule, as tested on a fibroblast cell line (A-1) the normal growth of which is dependent on the presence of ABPP in the medium (Saitoh et al., 1989, submitted). In addition, a small polypeptide, corresponding to the first 28 aminoacids of the amyloid β protein itself, was demonstrated to enhance neuronal survival, although at higher concentrations (Whitson et al., Science, 243:1488-1490, 1989). As a first step toward a better understanding of the molecular mechanisms underlying this phenomenon, we tested the biological activity of various domains of ABPP on A-1 cells and in transfection experiments. We engineered new plasmids that direct the expression of different regions of the ABPP. Two of these constructs consist of the N-terminal portion of ABPP including the Met initiation codon, some upstream untranslated sequences, and going down to the codon for Glu 305, with and without the 168 bp DNA fragment encoding the protease inhibitor domain of ABPP. Two other constructs are identical to those except that they extend down to the codon for Phe 600, which is the fourth amino acid of the amyloid β protein itself. These constructs were used to test the biological effects of the different domains of ABPP.

541.13

TEMPORAL EXPRESSION OF AMYLOID-BETA-PROTEIN mRNA IN HIPPOCAMPAL NEURONS *IN VITRO*. M.J. Strong*, A Svedmyr, R.M. Garruto, D.C. Gajdusek. LCNSS, NINDS, NIH, Bethesda, Maryland 20892

The origin of the amyloid-beta-protein deposited in the neuritic plaque of Alzheimer Disease is unknown. We examined the potential for a neuritic origin and have evaluated the temporal expression of amyloid precursor protein (APP) mRNA in developing fetal hippocampal neurons *in vitro*. Dissociated hippocampal neurons from New Zealand white rabbits at fetal day 29-30 were cultured in a chemically defined medium over confluent mature astrocytes. *In situ* hybridization with a biotinylated riboprobe transcribed from a cDNA encoding the amyloid precursor protein was performed at intervals to 30 days in culture. Age matched cultures were hybridized with a sense probe as controls. Prior to the development of features of neuronal maturity, APP mRNA could be localized to the neuronal perikarya and proximal neuritic tree. As the neurons reached maturity, signaled by the expression of all neurofilament subunit proteins and by the acquisition of morphological features of hippo-campal neurons *in vivo*, the APP mRNA localized to the perikarya alone. Our findings demonstrate the expression of APP mRNA in hippocampal neurons in vitro and also suggest that this expression is developmentally regulated. (*supported in part by the Medical Research Council of Canada)

541.14

RAPID INCREASE OF PAIRED HELICAL FILAMENT AND ß-AMYLOID (A4) IMMUNOREACTIVITY IN VERY MILD ALZHEIMER'S DEMENTIA. J.L. Price, P. Davis*, J.C. Morris*, D.L. White* and H. White* (SPON: L. Berg), Anat./Neurobiol. & Alzheimer Disease Res. Cntr., Washington Univ. Sch. Med., St. Louis, MO 63110

Tangles, plaques and immunohistochemical staining with antibodies against paired helical filaments (PAM; from Dr. D. Selkoe) and ß-amygdaloid (anti-A4, from Dr. C. Masters), and with the Alz50 antibody (provided by Dr. P. Davies), have been mapped and counted in non-demented and demented subjects, aged 59 to 87. The cognitive status of each case was assessed by premortem testing, and/or by a structured retrospective interview with a close relative. The distribution and density each marker was determined from serial sections through the ventral forebrain with the aid of a computerized microscope digitizer.

At least a few tangles are found in all of the cases, and there is a progressive increase in their density and extent, from non-demented cases to very mildly demented cases to severe cases. In non-demented cases there are similar densities of tangles, Alz-50 and PAM immunoreactive cells (A50-IC and PAM-IC). However, in very mildly demented cases PAM-IC have increased to about twice the density of tangles or A50-IC, suggesting a very rapid increase in the number of affected cells. In severely demented cases, the density of tangles has increased to levels comparable to the levels of PAM-IC in the very mild cases.

Plaques are rarely found in non-demented cases, but are present in high density in even the very mildly demented cases. The density and distribution of plaques is approximately the same with the Bielschowsky method and with anti-A4 immunohistochemistry (A4-IHC), although diffuse, 'fluffy' staining of amyloid material in superficial and middle cortical layers may be more prominent with A4-IHC.

Supported by NIH grants AG05681 and AG03991.

541.15

EXPRESSION OF β-AMYLOID PRECURSOR PROTEIN IN REACTIVE ASTROCYTES FOLLOWING NEURONAL DAMAGE. R. Siman, J.P. Card, R.B. Nelson and L.G. Davis. Medical Products Dept., The DuPont Co., Wilmington, DE. 19880-0400.

Although the beta-amyloid peptide is an established core component of neuritic plaques that accumulate in Alzheimer's disease, the mechanisms responsible for its deposition are not well understood. We now report that lesions of rat hippocampal neurons caused a time-dependent, long-lasting elevation of immunoreactivity for the beta-amyloid precursor protein (APP) in neighboring astrocytes, a cell type not normally expressing the protein in hippocampus. Neuronal damage produced by intraventricular kainate or colchicine injections or by stab wound all caused the aberrant APP accumulation. The increase represented astroglial expression of the protein rather than a scavenging of APP released by damaged neurons. Immunoelectron microscopy confirmed that APP-containing cells are reactive astroglia, both surrounding capillaries and within the neuropil. Antisera directed against either the amino- or carboxy-terminal of APP labelled reactive astroglia, consistent with expression of a full length precursor by these cells. The results demonstrate that neuronal damage stimulates APP expression in adult brain, and suggest that reactive astrocytes may be a source of the beta-amyloid that forms neuropathological plaques in Alzheimer's disease.

541.16

LOCALIZATION OF β-AMYLOID PRECURSOR PROTEIN IN PLAQUES AND REACTIVE GLIA IN HUMAN ALZHEIMER'S BRAIN. J.P. Card, R. Siman, E.J. Mufson[1] and L.G. Davis, Medical Products Department, DuPont Company, Wilmington, DE. 19880 and [1]Institute for Biogerontology Research, Sun City, AZ. 85372.

Antisera generated against two distinct domains of the β-amyloid precursor molecule (APP) were used to localize this protein in human brain. APP distribution in hippocampus, temporal cortex and visual cortex of Alzheimer's brain was compared to that observed in postmortem tissue from patients with no known neurological deficit. Immunohistochemical localization of APP with an antiserum against the first 20 amino acids of the beta peptide (#597-616 as described by Kang et al., '87) revealed extensive plaque distribution in circumscribed areas of Alzheimer's tissue which was not apparent in normal tissue. The regional plaque distribution with this antiserum correlated with that identified by Congo Red and thioflavin-S staining; however, the pathological deposition of amyloid identified immunohistochemically was far more extensive. In particular, exceptionally large numbers of plaques exhibiting diverse morphology were differentially concentrated in the superficial layers of temporal cortex and subiculum. Immunohistochemical analysis with an antiserum generated against the 20 amino acids (#619-638) at the C-terminus of the beta peptide also revealed immunoreactivity within reactive astroglia surrounding these plaques. Dual immunocytochemical labeling of APP and GFAP confirmed the astroglial identity of these cells and the preferential association with plaques. These data indicate that induction of amyloid in reactive glia may contribute to the neuropathology evident in Alzheimer's disease.

541.17

CHARACTERIZATION AND PARTIAL PURIFICATION OF THE β-AMYLOID PRECURSOR PROTEIN FROM RAT BRAIN. R.Lampe, L.G. Davis, J.P. Card and R. Siman. Medical Products Dept., The DuPont Co., Wilmington, DE. 19880-0400.

The beta-amyloid peptide is found as a core component of neuritic plaques in Alzheimer's disease, and is contained within a family of precursor proteins (APP) of predicted sequences from cDNA clones. However, APP is of unknown structure and function. We have raised antisera to a variety of peptide domains from this protein and used Western blot analysis to detect, characterize and partially purify APP from rat brain. Carboxy-terminal antibodies recognized a triplet of APP polypeptides of Mr~110-130 kD. These polypeptides were exclusively associated with membranes, but could be selectively extracted in low ionic strength buffer, a property characteristic of extrinsic membrane proteins. APP appears not to be glycosylated, since it neither bound to various types of lectin-conjugated Sepharose beads, nor was susceptible to glycosidase-mediated alteration in gel migration. APP was purified more than 2000-fold by ion-exchange chromatography, gel filtration, and heparin-agarose affinity chromatography. Through these procedures, two of the APP polypeptides co-purified, while the third was differentially separated by the anion exchanger.

541.18

PROTEOLYTIC PROCESSING OF BETA-AMYLOID PRECURSOR PROTEIN BY CALPAIN I. L.G. Davis, J.P. Card, R.P. Meade* and R. Siman, Medical Products Dept., The DuPont Co., Wilmington, DE. 19880-0400.

Since the beta-amyloid peptide is contained within a family of precursor proteins (APP), proteolytic processing of APP is required for beta-amyloid deposition into neuritic plaques. We have begun to examine mechanisms of APP processing, using two approaches. First, immunoblotting with APP antisera was used to assess the *in vitro* degradation of rat or human brain APP by a battery of purified proteases. Second, the localization of APP was compared with that of several proteases by immunocytochemistry. On Western blots, antibodies to the C-terminal domain of APP labelled three to four polypeptides in rat and human brain membranes, of Mr~110-135 kD. Both rat and human APP were exquisitely sensitive to a variety of proteases, including the calcium-activated protease calpain I, trypsin, papain, and cathepsin G. In particular, calpain I degraded APP under conditions in which few other proteins were affected. Throughout rat brain, calpain I and APP immunoreactivities were co-localized within the same neuronal populations, with especially high levels of each in olfactory bulb, layer 5 of neocortex, subiculum, globus pallidus, red nucleus, and cerebellar Purkinje cells and deep nuclei. Intraventricular kainate infusion, which causes rapid activation of hippocampal calpain I (Siman and Noszek, *Neuron*, 1988), produced a 22% decline in APP content by 24 hours, suggestive of *in vivo* degradation of APP by calpain I. By two weeks, substantial neuronal loss had occurred and both APP and calpain I immunoreactivities had appeared in the surrounding reactive astroglia. Collectively, these results indicate that calpain I may be involved in the normal and, perhaps, pathological processing of APP.

541.19

ELEVATION OF AN AMYLOID PRECURSOR mRNA IN ALZHEIMER'S DISEASE AND DOWN SYNDROME. R.L. Neve[1] and G.A. Higgins[2]. [1]Children's Hospital, Boston, MA 02115; [2]University of Rochester Medical Center, Rochester, NY 14642.

At least three different alternately spliced mRNAs encode three variants of the Alzheimer amyloid precursor protein (APP). The sequence of events leading to the aberrant proteolytic processing of the amyloid precursor(s) may be tied to a change in abundance of expression of one of the forms of APP. To learn more about the relationship of APP mRNA expression and the development of AD and Down syndrome (DS) neuropathology, we have undertaken a systematic analysis of APP mRNA expression in defined regions of normal and diseased brains. We have combined two methods of quantitative analysis: (1) Fixed tissue sections are used for direct quantification of APP mRNAs, using in situ transcription followed by polymerase chain reaction (PCR) amplification of the cDNA; and (2) adjacent sections are hybridized in situ with APP-specific oligonucleotide probes to confirm the PCR quantification and to define the distribution of cells showing altered expression of APP mRNAs. In the hippocampal formation, the expression of APP-751/770 is increased in the CA1 region and in the subiculum of both aged DS and AD individuals compared to controls. A similar increase in the ratio of APP-751 mRNA to APP-695 mRNA occurs in the basal forebrain in AD as compared with normal aged controls.

541.20

NERVE GROWTH FACTOR (NGF) REGULATION OF AMYLOID GENE EXPRESSION IN AGED RAT FOREBRAIN. Gerald A. Higgins[1], Rachael L. Neve[2], Karen S. Chen[3] and Fred H. Gage[3]. [1]University of Rochester Medical Center, Rochester, NY 14642; [2]Children's Hospital, Boston, MA 02115; [3]University of California at San Diego, La Jolla, CA 92029.

Deficits in the NGF-responsiveness of basal forebrain cholinergic neurons may contribute to pathological changes in the aged CNS. One gene whose expression appears to be regulated by NGF is the amyloid protein precursor (APP), which encodes the ß/A4 protein component of amyloid deposits in aged and Alzheimer's diseased brain. In order to understand the regulation of APP gene expression by NGF *in vivo*, we have initiated studies in the basal forebrain of adult and aged rats using *in situ* hybridization and quantitative mRNA analysis of different APP transcripts, NGF receptor (NGF-R) and choline acetyltransferase (ChAT) mRNAs. NGF or vehicle was infused into the striatum, and spatial memory was tested in a water maze task. In young adult rats, chronic NGF infusion produces robust increases in APP mRNA hybridization, NGF-R mRNA hybridization, NGF-R immunoreactivity, ChAT mRNA hybridization, and hypertrophy of ChAT mRNA-positive neurons. NGF treatment also increases the ratio of APP-695 mRNA to APP-751 mRNA in the basal forebrain. Aged rats with spatial memory deficits show increased levels of APP-751 mRNA in the forebrain as compared to aged non-impaired or young control rats. We are currently examining whether NGF treatment in aged animals can reverse changes in APP gene expression associated with behavioral impairment.

541.21

CHARACTERIZATION OF NEUROTOXICITY OF A FRAGMENT OF THE ALZHEIMER AMYLOID PRECURSOR IN PRIMARY RAT HIPPOCAMPAL CULTURES. L.R. Dawes and R.L. Neve. The Children's Hospital, Boston, MA 02115.

Conditioned medium (CM) from PC12 and NIH 3T3 cell transfectants expressing the carboxyterminal 105 amino acids (AB1) of the Alzheimer amyloid precursor (APP) is toxic to neurons in primary cultures of E18 rat hippocampus. Within 2-4 days following addition of 50% AB1 CM to the cultures, neurons in the cultures exhibit loss of phase brightness, roughening of soma, and disintegrating neurites; and a population of non-neuronal cells displaying normal morphology. The toxicity is dependent on both the density and the age of the cultures. At low plating density virtually all the neurons are killed, whereas the proportion of neurons surviving 4 days of exposure to AB1 CM increases at higher densities. Neurons cultured for one month in vitro are more resistant to the AB1 CM toxicity, and may be cultured for two weeks in the presence of AB1 CM with minimal cell loss (comparable to control cultures grown in the presence of CM from nontransfected cells). The glutatmate receptor antagonists kynurenate (1 mM) and 2-amino-5-phosphovalerate (APV, 100 uM) do not protect the neurons against the neurotoxicity of AB1 CM. Future studies are directed towards pharmacological interventions which may ameliorate the AB1 neurotoxicity, as well as towards the purification of the neurotoxic agent.

541.22

A RELATIONSHIP BETWEEN EXTRACELLULAR LOCALIZATION OF LYSOSOMAL PROTEINASES AND AMYLOID DEPOSITION IN ALZHEIMER'S DISEASE. A. M. Cataldo* and R. A. Nixon. McLean Hospital, Harvard Medical School, Belmont, MA 02178

The importance of proteolysis in the formation of amyloid in Alzheimer's disease (AD) is becoming increasingly evident. Our previous studies have shown that antibodies to the lysosomal proteases, cathepsin D (CD) and cathepsin B (CB), are particularly abundant within "at-risk" neuronal populations of AD brains and are present extracellularly in senile plaques (SP) as well. We found that dying neurons, which display an abnormal accumulation of lysosomes, are a major source of these enzymes. To investigate the relationship between localization of cathepsin immunoreactivity and the amount of amyloid deposited in the SP of AD brains, we analyzed tissue sections immunocytochemically with antisera to human CB and CD and histologically using Bielschowsky silver stain and thioflavin S. Intense CD and CB immunoreactivities were consistently associated with SP that exhibited moderate or marked thioflavin positivity. These SP, identified as "classical" plaques, were most abundant within cortical layers III and V and layer II of the hippocampus. Silver positive "primitive" type SP, which were distributed throughout all cortical and hippocampal laminae (particularly the molecular layer), did not contain prominent thioflavin staining, and were not highly immunostained with cathepsin antibodies. Our results suggest a strong relationship between prominent amyloid deposition extracellularly and the intense localization of lysosomal proteinases. We propose that lysosomal enzymes, released into the extracellular space from dying neurons, may contribute to the generation of amyloid and amyloid-related proteins within the SP of AD brain. Supported by NIA and AFAR.

541.23

A4 AMYLOID PROTEIN IMMUNOREACTIVITY IN NEUROFIBRILLARY TANGLES AND TERMINAL ZONES OF THE HIPPOCAMPAL FORMATION IN ALZHEIMER'S DISEASE. B.T. Hyman, G.W. Van Hoesen, C.L. Masters* and K. Beyreuther*. Dept. of Neurology, Harvard Medical School., Boston, MA; Dept. of Anatomy, Univ. of Iowa, Iowa City, IA 52242; Melbourne, Australia; Heidelberg, Fed. Rep. Germany.

A4, the core protein of amyloid neuritic plaques (NPs) in Alzheimer's disease, is thought to be a transmembrane synaptic protein. Whether A4 is also a component of neurofibrillary tangles (NFTs) is controversial. Because specific neurons in the hippocampal formation are known to undergo degeneration, those neurons and their terminal zones were examined for A4 immunoreactivity. In minimally-fixed frozen tissue sections several novel aspects of A4 immunoreactivity were noted: (1) diffuse A4 deposits occurred in many areas that did not contain NPs as shown by thioflavin S or silver stains, including layer III of entorhinal cortex, amygdala, and the basal ganglia; (2) A4 immunoreactivity occurred in a pattern consistent with predicted terminal zones; and (3) some NFTs are A4 immunoreactive, particularly those of the "tombstone" variety (i.e. that no longer contain stainable Nissl substance and are Alz-50 and tau negative). These results support the hypotheses that A4 is extensively distributed, is deposited in terminal zones of degenerating neurons, and is associated with neurons that develop NFTs. (Supported by: NS 14944, PO 19632.)

541.24

DIFFERENTIAL LABELING OF ALZHEIMER DISEASE SENILE PLAQUES BY ANTIBODIES TO DISTINCT REGIONS OF THE ß AMYLOID PRECURSOR PROTEIN (ßAPP). C Joachim, D Games+, P Ward+, T Henriksson+, D Platt, D Schenk+, D Selkoe. Harvard Med Sch, Boston, MA, and Athena Neurosciences+, San Francisco, CA.

Senile plaques showing a spectrum of morphologies in the cerebrum are known to be labeled by antibodies to synthetic or purified ß amyloid protein (ßAP), also called A4. We recently showed that antibodies to the C-terminus and N-terminus of ßAPP recognize a subgroup of cerebral plaques. We now compare several antibodies raised to non-ß regions of ßAPP to ßAP antibodies; the latter detect virtually all plaques. We find that: 1) The non-ß region antibodies label a minority of cerebral plaques, in which they stain discrete globular and granular structures. 2) Almost all plaques detected by the non-ß region antibodies contain dystrophic neurites. 3) Double-labeling using a tau or neurofilament antibody with a non-ß region antibody (αB5 to a recombinant protein of ßAPP residues 444-592) showed that some αB5-positive structures were not recognized by tau or neurofilament antibody though neurites labeled by the latter antibodies were usually in close proximity. 4) Amyloid cores and vascular amyloid were not detected by the non-ß region antibodies. We conclude that non-ß region antibodies appear not to detect ßAP deposits per se but only neurite-containing plaques, and within them, may label dystrophic neurites themselves. In diffuse plaques and vascular amyloid, the amyloidogenic fragment appears not to contain the N- and C-termini of ßAPP.

541.25

MEMBRANE-ASSOCIATED AND SOLUBLE FORMS OF ß-AMYLOID PRECURSOR PROTEIN (ßAPP) IN HUMAN TISSUES & FLUIDS. M. Podlisny*, A. Gronbeck*, A. Mammen*, M. Schlossmacher*, M. Palmert*, D. Chin*, S. Younkin°, T. Oltersdorf+, & D. Selkoe, Harv Med Sch, Boston, °Case W. Res, Clvd, OH & +Athena NS, San Fr, CA

Using tissue fractions & cDNA-transfected cells, we previously showed that ßAPP occurs in mammalian tissues as a group of ~110-135 KD membrane-associated proteins. Since a ~40-residue hydrophobic fragment of ßAPP forms the ßAP (A4) deposits in AD & these occur outside cells, the processing of ßAPP into such fragments should be elucidated. In both human brain & transfected cells, ßAPP C-terminal antisera detect an ~11 kD membrane-associated protein that appears to be a favored & stable proteolytic fragment containing the C-terminus & presumably ßAP. ßAPP$_{695}$-transfected cells produce more 11 kD & other low MW fragments than do ßAPP$_{751}$ transfectants, suggesting that the KPI domain can inhibit breakdown of ßAPP itself. The 11 kD fragment is found in kidney, adrenal & other non-neural tissues. It is also produced in brains of rats & mice, which don't develop ßAP deposits. Thus, processing events besides production of the 11 kD fragment are needed for ß-amyloidosis. Separate studies with N-terminal antibodies reveal ~105-120 kD soluble ßAPP forms in human plasma that comigrate with soluble forms previously described in CSF. These appear to lack the C-terminus, suggesting that the large N-terminal portion of ßAPP is released into extracellular fluids following cleavage near the transmembrane region. The relevance of these various forms to ß-amyloidosis will be discussed.

541.26

SOLUBLE DERIVATIVES OF THE ß AMYLOID PROTEIN PRECURSOR: PURIFICATION FROM CEREBROSPINAL FLUID AND SEQUENCE ANALYSIS. M.R. PALMERT*, T.L. ROSENBERRY*, AND S.G. YOUNKIN. Inst. of Pathology, Div. of Neuropathology, and Dept. of Pharmacology, Case Western Reserve Univ. Sch. of Med., Cleveland, OH 44106.

The amyloid deposited in Alzheimer's disease (AD) is composed primarily of a small, 4.2 kDa protein (ßAP) that is derived from the ß amyloid protein precursor (ßAPP). In previous studies, we used antisera to synthetic ßAPP peptides to identify (i) a set of ~110-135 kDa membrane-associated proteins that represent full-length forms of the ßAPP and (ii) soluble ~105 and ~125 kDa derivatives of the ßAPP that lack the COOH-terminus of the full length, membrane-associated forms. In this study, we have confirmed our identification of the soluble ßAPP derivatives by co-purifying the ~105 and ~125 kDa proteins from human cerebrospinal fluid (CSF) by ammonium sulfate fractionation, FPLC using a Mono Q column, and preparative SDS-PAGE. The two proteins were then separately excised from the gel, eluted, subjected to SDS-PAGE, transferred to Immobilon, and sequenced. Nineteen of the 20 amino acids at the NH2-terminus of the more abundant ~105 kDa protein were identified, and all 19 of these amino acids were identical to those predicted from the published ßAPP cDNA sequence. Eleven of the 13 amino acids at the NH2-terminus of the ~125 kDa protein were also detectable, and all 11 were identical to those predicted. It is possible that the soluble ßAPP derivatives identified in this study are generated by cleavage at either the NH2- or COOH-terminus of the ßAP, which is encoded as an internal peptide in the full-length ßAPP molecule. Since these proteins can be readily purified from CSF, it should be possible to characterize their COOH-termini, identify the specific site at which the ßAPP is cleaved during their generation, and determine if this site is relevant to ßAP formation.

541.27

ALZHEIMER'S DISEASE DOES NOT ALTER THE PROPORTION OF HIPPOCAMPAL NEURONS WITH APP-695 OR APP-751 mRNA. S.A. Johnson and C.E. Finch. Andrus Gerontology Center, Univ. Southern Caliifornia, Los Angeles, CA. 90089-0191.

Northern blot analyses have shown a selective decrease of the amyloid precursor protein mRNA which lacks a Kunitz protease inhibitor motif (APP-695 mRNA) in Alzheimer's disease (AD) cortex and hippocampus. This selective APP-695 mRNA reduction could be due to: 1) a loss of neurons which only express APP-695 mRNA; 2) decreased stability of APP-695 mRNA (or increased stability of APP-751 mRNA); 3) an alteration of precursor APP mRNA splicing leading to decreased APP-695 mRNA prevalence. We have addressed the first alternative by high criterion in situ hybridization of AD and control hippocampus with APP-695 and APP-751 mRNA specific cRNA probes. Serial sections were hybridized separately to each probe. Every nucleolated pyramidal neuron with a signal above local background levels was counted throughout the Cornu Ammonis subfields of 7 AD and 4 CTL specimens. There are equivalent numbers of APP-695 and APP-751 transcript specific neurons in AD and control specimens. This analysis suggests that a specific loss of neurons which only express APP-695 mRNA does not occur in AD hippocampus, and is not a mechanism to explain the selective reduction of APP-695 mRNA. These studies were supported by grants to CEF (AG07909) and SAJ (ADRDA, Investigator Initiated Research Grant).

AGING II

542.1

PROTEIN KINASE CHANGES IN AGED ALUMINUM-TREATED RATS. C.B. Caputo*, L.A. Sygowski*, C.W Scott*, G.V.W. Johnson, W.F. Brunner* and A.I. Salama (SPON: R.D. Krell). Dept. of Pharm., ICI Pharmaceuticals Group, ICI Americas Inc., Wilm. DE 19897 and Univ. of Alabama, Birmingham, AL 35294.

Four protein kinases that copurify with neurofilaments (NF) and microtubules (MT) were assayed in brain and spinal cords of young (1 mo) and aged (26 mo) rats. The activities of 3 of these kinases were substantially decreased in aged rat tissue. These 3 were cAMP-dependent kinase and a cofactor-independent (N1) kinase associated with NF and a cofactor-independent MT kinase. $Al_2(SO_4)_3 \cdot 16H_2O$ was administered at 0.3% (Al) in drinking water for 1 mo to young and aged rats. Another group of aged rats was given 0.3% Al for 1.5 yr (from 1 yr of age). All kinases were significantly elevated in aged rats after Al treatment. After 1 mo and 1.5 yr cAMP kinase was elevated 31 and 167%, respectively, N1 kinase by 67 and 312%, and MT kinase by 660 and 420%. Ca-calmodulin dependent kinase (with NF) was elevated only after 1 mo, by 138%. Al treatment did not affect the ability of NF proteins to incorporate PO_4 by control kinases. No changes in kinases from young rats were observed after Al although histopathological changes in spinal cord occurred, suggesting that some Al did cross the blood brain barrier. It is concluded that aged rats showed higher sensitivity than young rats to an Al effect which results in elevated kinase activities.

542.2

TRANSPLANTATION AND SURVIVAL OF NEURONS IN THE BRAIN OF OLD HAMSTERS. E. E. Morrison and R. M. Costanzo. Department of Physiology, Medical College of Virginia, Richmond, VA 23298-0551.

In previous studies olfactory neurons have been successfully transplanted into the brain of neonate or young adult host animals. The present study was undertaken to determine if the "old" brain could serve as a site for the transplantation of neurons. Old hamsters (1-2 years) were used as host animals. Olfactory neurons were obtained from the septal region of neonate hamsters and transplanted into frontal or parietal host cortex. For periods up to 115 days transplanted neurons survived and continued to develop in the "old" host brain environment. Mitotic figures were observed among transplanted neurons and developing axons originating from these neurons grew into the host cortex. The results of these experiments demonstrate that olfactory neurons are capable of surviving and developing even when transplanted into old host brain environment. These results suggest that regeneration and nerve cell replacement may be possible in the aging mammalian brain. Supported by Jeffress Research Grant J-122 to EEM and NIH Grant-NS16741 to RMC.

542.3

AGE-DEPENDENT RESPONSE OF NEURITE OUTGROWTH FROM GANGLIA EXPLANTS TREATED WITH PHORBOL ESTERS.

L. Hsu, A.Y. Jeng* and K.Y. Chen*, Biol. Dept.,Seton Hall Univ.,S. Orange NJ 07079; Research Dept., Pharma. Div., Ciba-Geigy Corp., Summit, NJ 07901; Chem. Dept., Rutgers Univ., Piscataway, NJ 08854

We have shown 12-0-tetradecanoylphorbol 13-acetate (TPA) and other activators of protein kinase C (PK-C) to be potent promoters of neurite outgrowth from explanted sensory ganglia of embryonic chicks. This response to TPA was both age- and dose-dependent. At 20 ng/ml, TPA was effective only on explants from young embryos (9d) while those from older embryos (14-16d) required up to 500 ng/ml to produce neurites. Explants from embryos beyond 18 d of age did not respond to TPA treatment. This decreased response to TPA to develop neurites in older ganglia was not related to decreased levels of PK-C activity. In contrast, ganglia from older embryos contained higher levels of PK-C activity. In addition, the lack of response was not due to the loss of receptors for TPA since phorbol ester binding was maximal in older ganglia. These results suggest that while neurite outgrowth requires the activation of PK-C, additional factors other than receptor binding and PK-C activity are also needed for this process.

(Supported by NS21262)

542.4

THE AGE-DEPENDENT DECREASE IN CORTICAL ACETYLCHOLINE (ACh) RELEASE IN THE RAT IS RESTORED BY PHOSPHATIDYLSERINE.

F. Casamenti*, M.G.Vannucchi* and G.Pepeu. Dept. Pharmacology University of Florence, 50134 Florence, Italy.

The effect of phosphatidylserine (Ps) (15 mg/Kg i.p. for 7 days) on ACh release was investigated "in vivo" and "in vitro". On the last day of treatment, a transverse microdialysis tubing was stereotactically inserted through the parietal cortex (C.F.Wu et al.Neurobiol.Aging,9:357,1988). One day later the tubing was perfused with eserinized Ringer solution and ACh content of 20 min samples was detected and quantified by HPLC. ACh release was 328±16 fmol/min in 4 month-old rats, 215±11 (n=8,P<0.01) in untreated, and 316±13 in Ps-treated 19 month-old rats. Ps had no effect in young rats. In other rats cortical slices were prepared at the end of Ps treatment, incubated with [^{3}H] choline, superfused with eserinized Krebs solution and electrically stimulated (F.Pedata et al. Neurobiol.Aging, 4:31,1983). Total evoked ACh release and its specific activity were 22 pmol/min and 0.7% respectively in young, 12.7 and 0.6% (n=5, P<0.01) in untreated, and 20.1 and 0.3% in Ps-treated rats. This finding suggests that Ps treatment enhances the supply of endogenous choline for ACh synthesis. (Supported by CNR grants).

542.5

EFFECTS OF AGING ON CGRP AND NON-CGRP NEURONS IN THE RAT TRIGEMINAL GANGLION.

M.A. Biedenbach[1], D.C. Herbert*[2], and D.N. Kalu*[1]. Univ. of TX Hlth Sci. Ctr., [1]Dept. of Physiol., and [2]Dept. of Cell. Struct. Biol., San Antonio, Texas 78284.

This study is part of a larger project that aims to compare the expression of the calcitonin gene as function of age in a highly proliferative tissue (the thyroid) and in a postmitotic tissue (the trigeminal ganglion). In the thyroid we found marked increases with aging in C-cell density, calcitonin, CGRP and their mRNA's (J. Geront.: Biol. Sci. 43:B125 and unpubl. observat.).

The purpose of the current study was to determine the effects of aging on CGRP and nonCGRP neurons in the trigeminal ganglion, where the calcitonin gene produces only CGRP. F344 rats (ages 6, 12, 24 months) were anesthetized, the trigeminal ganglia removed and serial sections prepared. The sections were immunocytochemically stained for CGRP and counterstained with H & E. In selected sections all neurons and nuclei were traced, coded as CGRP or non-CGRP and entered into computer files for morphometric analysis. The results showed, in young adult rats CGRP cells are 20-30% of all neurons with a mean soma diameter of 20.6µ, range 11-41µ, nucleus:soma diameter ratio 0.49. For nonCGRP cells equivalent values were 22.8µ, 11-47µ, 0.47, respect. In old rats the only change noted was a small increase in soma size of all neurons, i.e., aging changes in this postmitotic tissue are minor as compared to the thyroid gland.

542.6

AGE AFFECTS THE REPRODUCTIVE RESPONSE TO MELATONIN BUT NOT THE GnRH NEURONAL SYSTEM IN THE DJUNGARIAN HAMSTER.

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During reproductive development, short days or timed melatonin treatments inhibit gonadal growth and result in a decrease in total number of GnRH neurons. We hypothesized that as in peripubertal males, hamsters throughout adulthood would similarly respond to timed melatonin treatments. Adult (3 mo) and aged (>12 mo) male Djungarian hamsters in 16L:8D (L off 1800 h) were injected daily at 1600 h with melatonin (2.5 µg/0.2 ml sc), a treatment known to regress the testes. As controls, adult and aged males received daily melatonin at 0900 h or saline at 1600 h. After four weeks, animals were perfused through the heart, and the testes removed and weighed. Every brain section (60 µm) from the anterior commissure decussation to the ventromedial hypothalamus (the location of the majority of GnRH cell bodies in this species), was processed for GnRH immunocytochemistry (LR-1 gift of R. Benoit). GnRH positive perikarya were categorized and counted. For saline and melatonin treated controls, testes weights were similar in adult and aged hamsters (about 600 mg). Four weeks of melatonin at 1600 h significantly reduced testes weight in adults (144 ± 30 mg, n=7), however, aged hamsters were less responsive (360 ± 83 mg, n=8). For GnRH neurons, the total number of perikarya was the same in all groups (about 140/brain), with a ratio of unipolar to bipolar cell bodies of about 3:2. Moreover, GnRH neuron numbers within the preoptic and hypothalamic areas remained unchanged irrespective of treatment. These results indicate that the reproductive system responds to timed melatonin injections from the onset of puberty through old age. Although this response is diminished in aged hamsters, a relatively stable population of GnRH neurons appears unaffected by melatonin or by the aging process. (Sup. NIH HD22479)

542.7

AGE-RELATED BEHAVIORAL AND NEUROCHEMICAL ALTERATIONS: LACK OF CHRONIC CALORIC RESTRICTION AMELIORATIVE EFFECTS.

F.M. Scalzo, R.R. Holson, S.F. Ali, P.A. Sullivan-Jones*, R.W. Hart*. Division of Reproductive and Developmental Toxicology, The National Center for Toxicological Research, Jefferson, Arkansas 72079-9502.

Reports of alterations in behavior and neurochemistry during aging in rodent and other mammalian species have been mixed. Some investigators report alterations, while others report no changes in learning and dopamine and cholinergic receptor binding during aging. Caloric restriction has been shown to extend life-span, reduce both tumor incidence and the magnitude of behavioral and neurochemical alterations reported in ad lib-fed aged animals. The present studies determined if there were age-related changes in complex maze performance in the Brown Norway rat, dopamine and muscarinic cholinergic binding in the B6C3F1 mouse, and the interaction of caloric restriction with these changes. Brown Norway rats and B6C3F1 mice were fed ad lib or calorie restricted diets (60 % of ad lib) from 14 weeks of age. Complex maze performance was impaired in rats at 14 and 23 months compared to 7 months, while muscarinic cholinergic binding was decreased at 30 months of age. Caloric restriction did not ameliorate either of these changes. The results suggest that caloric restriction does not alleviate age-related alterations in behavior or muscarinic binding in these two species.

542.8

HOMOLOGOUS mRNA FOR TESTICULAR HEAVY CHAIN DYNEIN IN EPENDYMAL CELLS AND NEURONS.

N.A. Seniuk*, W.G. Tatton, A.T. Garber*, and G.H. Dixon*, Physiology Dept., Univ. of Toronto, Toronto and Medical Biochemistry Dept., Univ. of Calgary, Calgary, Canada

Dynein proteins are known to be the ATP-ase "motors" for cilial and flagellar movement and recently have been shown to mediate neuronal retrograde axonal transport (Vallee et al. 1989 #1.). A cDNA from trout testis for heavy chain dynein (hcD) has been found in high concentration in brain tissue on northern blots (Garber et al. 1989 #2.). We have carried out immunocytochemistry for hcD and alpha tubulin (aT) polyclonal antibodies (pabs) and in situ hybridization for hcD and aT mRNAs in trout and mouse brain. Both procedures used an avidin-HRP chromogen system. Immunoreaction was strongly positive for the hcD and aT pabs in both the somata and axons of neurons and the subcilial regions of ependymal cells but not in glial cells. In the larger diameter trout axons, linear arrays of hcD immunoreaction parallel to the axonal long axis were evident. Biotinylated RNA probes (incorporating biotin-11-UTP) complementary to hcD and aT message revealed dense hybridization in the somata of neurons and in ependymal cells (probes with message sequences were negative). Stingencies of up to 50% formamide and 50 deg. C were used. The subcellular concentrations of hcD and aT probes differed in that hcD probes had a perinuclear preponderence. In trout neurons there was some nuclear hybridization that excluded the nucleolus. Computer densitometric analysis showed a relatively greater density of hcD hybridization in neurons with long axons than those with local ramifications. The immuncytochemistry and in situ hybridizations suggest common hcD epitopes and similarity of hybridizable base sequences for hcD in testicular cells, neurons and ependymal cells and mRNA and revealed localizations that are consistent with the "motor" functions of dynein.

(Supported by MRC Canada) #1. TINS 12,66-70, #2. EMBO in press.

542.9

AGE-INDUCED CHANGES IN SINGLE LOCUS COERULEUS BRAIN GRAFTS GROWN IN OCULO: AN IN VIVO ELECTROCHEMICAL STUDY. G.A. Gerhardt[1,2], A.-Ch. Granholm[3] and M.R. Palmer[2]. Depts. of Psychiatry[1] and Pharmacology[2], Univ. of Colorado Health Sciences Center, Denver, CO 80262 and Dept. of Cell Biology[3], Univ. of Linköping, Sweden.

Grafts of fetal rat tissue transplanted to the anterior eye chamber of a host animal are an excellent tool for studying maturation, aging and function of CNS tissues that are often difficult to study in intact animals. In the present study, fetal Sprague-Dawley locus coeruleus (LC) grafts were transplanted into the anterior eye chamber of host rats and were studied at 4-6 months (control) or 24-26 months (aged) after grafting. High-speed in vivo electrochemical measurements were used to characterize the potassium-evoked synaptic overflow from norepinephrine (NE)-containing cells in both aged and young brain grafts. Potassium-evoked responses were consistently seen to be attenuated in the aged grafts as compared to the young control grafts. However, as we have previously demonstrated, the NE content of aged single LC grafts is nearly identical to the young LC levels. These data suggest that the age-induced change in the potassium-evoked overflow of NE seen in LC brain grafts is likely not solely related to changes in storage and synthesis of NE. Supported by USPHS grants AG06434, AG00441, AA00102 and AA05915.

542.10

AGE-RELATED ALTERATION IN THE DOPAMINE-RECEPTOR ADENYLATE CYCLASE SYSTEM OF THE RAT STRIATUM. T. Saito, H. Ikeda, H. Ozawa, Y. Hatta, S. Hatta, and N. Takahata. Departments of Neuropsychiatry and Pharmacology, Sapporo Medical College, Sapporo 060, Japan.

The effects of age on dopamine receptor adenylate cyclase (AC) coupling were examined in rat striatum. There was no difference in [^{3}H]SCH 23390 binding to dopamine receptor in the membrane of between 24 month old rats (aged rats) and 2 month old rats (controls). Basal and Mn-stimulated AC activities were reduced in aged rats compared with those in controls. GppNHp-stimulated and dopamine-stimulated AC activities were also lower in aged rats compared with those in controls. The percent decrease in the GppNHp-stimulated AC activity was greater than in the Mn-stimulated AC activity. However, neither the EC_{50} value for GppNHp activation of AC nor the AC activity in the presence of a saturated level of GppNHp was altered in aged rats. There was no appreciable difference between the pattern of autoradiograph of ADP-ribosylation by cholera toxin obtained from aged rats and that from controls. The AC activity in the membrane preloaded with GppNHp in aged rats was 70% less than that in controls. Furthermore, the forskolin binding to striatum, analyzed from [^{3}H]forskolin autoradiograph, was significantly decreased in aged rats. Our results indicate that the decreased AC activity in aged rats may be due to the functional uncoupling of Gs-protein with catalytic unit and the reduced catalytic unit activity.

542.11

AGE-RELATED DECLINE IN RESPONSE TO NICOTINE: CORRELATION WITH A DECREASED SUBPOPULATION OF NICOTINIC RECEPTORS IN RAT STRIATUM. D.W. Schulz, G.A. Kuchel* and R.E. Zigmond. Dept. Biol. Chem. and Molec. Pharmacol., Harvard Medical School, Boston, MA 02115.

Studies of age-related changes in central cholinergic receptors have focused largely on muscarinic receptors. We have investigated the effects of aging on nicotinic receptors in the rat striatum using both functional and binding assays. As an assay of a functional response to nicotinic agonists in aged rats, we examined the ability of nicotine to stimulate endogenous dopamine release from striatal slices of 3 and 24 month old male Fischer 344 rats. We also measured the binding in striatum of ^{3}H-nicotine and ^{125}I-labeled neuronal bungarotoxin (NBT), a peptide that has been shown to block nicotinic function in various peripheral and central neuronal preparations.

In 3 month old rats, the release of dopamine was increased 168±33% above basal levels following a 15-second period exposure to nicotine (50 uM), while the increase in slices from 24 month rats was only 52±14% ($p<.01$). When 16 mM KCl was used to evoke dopamine release, no statistically significant difference between the two groups was observed. The striata of aged animals had 19% fewer sites labeled by 10 nM ^{3}H-nicotine than did the 3-month animals, a difference that was not statistically significant. However, when the binding of ^{125}I-NBT (3 nM) was examined with and without a high concentration of nicotine (100 uM), a 4-fold greater number of sites was found in 3 month old than in 24 month old rats ($p<.02$). The results indicate that there is a decreased functional response to nicotine observed in the striatum of aged animals, and at least part of this decrement may result from a decline in a subpopulation of nicotinic receptors that is labeled by ^{125}I-NBT.

[Supported by NS12651, MH00162, and a Fellowship Award from the Pharmaceutical Manufacturers Association Foundation].

542.12

EFFECT OF LONG-TERM CALORIC RESTRICTION ON ARTERIAL PRESSURE, HEART RATE AND PLASMA NOREPINEPHRINE CONTENT. H. Bertrand*, C. Stacy*, and J.T. Herlihy* (SPON: T. Mikiten). Univ. of TX Health Science Center, Dept. of Physiology, San Antonio, TX 78284-7756.

One current view of the aging process holds that aging is a hyperadrenergic state characterized by elevated sympathetic nervous system (SNS) activity. Life-long caloric restriction inhibits many age-related changes but its effect on the SNS has not been extensively studied. The present study examines the effect of chronic food restriction on physiological parameters known to be under the control of the SNS. The femoral artery and vein of 12 month old ad libitum fed (Group A) and food restricted (Group R) male Fischer 344 rats were cannulated. Blood pressure measurements and blood samples were obtained via the arterial cannula and drugs were infused via the venous cannula in awake, unanesthetized rats. The basal heart rate of Group R was lower than Group A, but no difference in mean arterial pressure was observed. When subjected to a hypotensive stress (nitroprusside infusion) both groups attained the same maximum heart rate. However, the response to graded nitroprusside infusions was greater in Group R than Group A. No differences in the basal plasma norepinephrine or epinephrine concentrations were observed between the two groups. These results suggest that food restriction 1) enhances the sensitivity of the baroreceptor reflex and 2) has no effect on the basal SNS activity. (Supported by NIA Grant #AG-01188)

542.13

Age-Related Expression of HSP-70 mRNA in Brain of Heat Stressed Rats. M.J. Blake, J. Fargnoli*, D. Gershon* and N.J. Holbrook*. Lab. Mol. Gen., Gerontol. Res. Cntr., Baltimore, MD 21224.

Functional deficits in neurotransmitter/hormone systems have been shown to contribute to an impaired ability to respond to physiologic stress with senescence. At the molecular level, recent studies have shown an age-related reduction in the stress-induced expression of heat shock proteins (HSPs) in cell culture. In this study we investigated age-related alterations in HSP 70 mRNA induction in brains from young (5 mo.) and old (24 mo.) male Wistar rats exposed to heat stress for various times. RNA was isolated from whole rat brains and assayed for HSP 70 expression by northern analysis. At all conditions young animals showed consistently higher HSP 70 mRNA induction relative to old animals. However, this age-related difference corresponded to a similar difference in core body temperatures (CBT) attained by young and old individuals in response to heat exposure. Young and old animals reaching equivalent CBTs displayed similar induction. The reduction of HSP 70 mRNA expression by heat in aged rat brain appears to result from a deficit in metabolic heat generation rather than an intrinsic alteration in HSP 70 regulation.

542.14

AGE-RELATED DECLINE IN SYMPATHETIC SPROUTING IS PRIMARILY DUE TO DECREASED TARGET SUPPORT. K.A. Crutcher, Dept. of Neurosurgery, University of Cincinnati Medical Center, Cincinnati, Ohio 45267.

Neuronal plasticity following CNS injury has generally been found to decrease with age. This is also true of the growth of sympathetic axons into the rat hippocampal formation following septohippocampal denervation (Scheff et al., Science,'78). To determine whether this decrease is due to reduced responsiveness of aged sympathetic neurons or to less support of sprouting by the aged hippocampal formation, the superior cervical ganglion was removed either bilaterally or unilaterally from young (3-4 month old) and old (24 month old) Fischer 344 female rats and transplanted to the dorsal hippocampal formation of either a young or old animal with or without a medial septal lesion. The transplant animals were examined after four weeks using catecholamine fluorescence and acetylcholinesterase (AChE) histochemistry. Essentially no innervation was observed in the absence of septal denervation, confirming previous work by Björklund and Stenevi (Brain Res., '81). With septal denervation, as evidenced by AChE depletion, the extent of sympathetic neuronal survival and hippocampal innervation depended more on the age of the host than the age of the donor. Thus, both young and aged ganglia showed poorer survival and less fiber growth into aged hippocampal tissue compared to young hosts. The topography of sprouting was similar regardless of host or donor age. The possibility that the reduced innervation in aged hosts was due to systemic factors, e.g. steroids, seems unlikely since there was always extensive regeneration within the transplant itself regardless of host age. Since NGF mRNA and NGF protein levels have been reported to be decreased in the aged rat hippocampal formation (Larkfors et al, Brain Res.,'87), an age-related reduction in target trophic support may account for the reduced sprouting response in aged animals. (Supported by NIA grant #AG-07691.)

542.15

AGE-RELATED COMPARISONS IN DETOUR MAZE PERFORMANCE USING A LEARNING SET PARADIGM WITH RATS.

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A shock avoidance maze task provided within subjects analysis of age-related performance differences using a learning set approach. After training to avoid footshock in the straight alley (SA) portion of the maze, male rats aged 6 (Y), 16 (M), 22 (A) mo were trained over 3 mo in a problem-solving task. Three pairs of U-shaped alleys extended to each side of the SA. Clear barriers could be placed in the runway between any detour pair to force the rat to go right or left. One of the pair was blocked at the end of the detour alley. On forced trails, entrance to one side of a detour pair was closed; on choice trials, entrances to both sides were open. Problem complexity was manipulated by increasing number of detours required (1-D 2-D 3-D). Six problems were given weekly, 1 problem/day, 14 trials/day (2 sample, 5 choice, repeated twice). A weekly 85% correct choice-trial criterion was required before moving to the next level of complexity. Y and M rats attained criterion within a wk on 1-D problems, 2 wk on 2-D, and 1 wk on 3-D. The A rats required extensive training on both 1- and 2-D problems, attaining criterion on 1-D but not on 2-D. On all measures (errors, correct trials, sample and choice runtimes), Y and M rats differed significantly from A rats but not from each other. After a 3-mo interval when M rats were 22-mo old, testing was repeated. At all complexity levels, performance during relearning was significantly more accurate than during acquisition. Runtimes remained unchanged. Because of improved choice accuracy by now-aged rats trained 6 mo earlier, memory function appeared unchanged, which differs from the cross-sectional perspective, perhaps because of previous experience or differences in health between cohorts.

542.16

INFLUENCE OF AGE ON THE MODULATION OF NOREPINEPHRINE RELEASE IN THE RAT TAIL ARTERY J. Buchholz* and S.P. Duckles.* Dept. of Pharmacology, Coll. of Medicine, Univ. of California, Irvine CA. 92717.

An established model of aging, the F-344 rat, was used to study the influence of age on modulation of stimulation evoked norepinephrine (NE) release. Release of NE and tissue NE content were measured in isolated perfused tail artery of 6, 12, 15, 20 and 27 month old rats. Tissues were stimulated transmurally with 3 min trains at 8 Hz. Norepinephrine was quantitated by HPLC with electrochemical detection. Spontaneous NE release was below the level of detection (33 pg/min). Tissue NE content was significantly less in the 6 month old animals as compared to the 12 month old animals. Stimulation evoked fractional NE release in the presence of deoxycorticosterone (DOC 10^{-5} M) and cocaine (COC 10^{-5} M) was altered with age so that 27 and 20 month old animals released significantly more NE than either 12 or 6 months. At 15 months significantly more NE was released than 6 months. Assessment of prejunctional α_2 adrenergic receptor activation was done in the presence of DOC+COC and yohimbine (10^{-7} M). Prejunctional α_2 adrenoreceptor activation was significantly reduced in 12, 15, 20 and 27 month old animals as compared to 6 months. Stimulation evoked NE release increases with age, and prejunctional α_2 adrenergic receptor activation decreases with age, although no progressive change with age in NE content was observed. *NIH Grant #AG06912*

542.17

Norepinephrine (NE) Stimulates Adenosine 3',5'- Monophosphate (cAMP) Production in the Olfactory Bulbs (OB) of Aged But Not Young Male Rats. T. Mencio-Wszalek, D.E. Dluzen and V.D. Ramirez. Department of Physiology and Biophysics, University of Illinois, Urbana, IL 61801.

In the present experiment, OB from young (less than 100 days of age) and aged (greater than 500 days of age) male rats were used. Samples from homogenized OB tissue extracts were incubated with one of two different dilutions of NE and levels of cAMP were subsequently analyzed by radioimmunoassay. cAMP production was inhibited to a similar degree when OB homogenates from both young and aged rats were incubated with 10^{-5}M NE. In contrast, only OB homogenates from aged rats exhibited significant ($p < .002$) stimulated cAMP production as compared to young when incubated with 10^{-7}M NE.

Mean % Change from Basal cAMP Production (measured in picomoles cAMP produced/mg protein/minute)

NE Concentration	Young(5)	Aged(6)
10^{-5} M	-81 ± 11	-68 ± 6
10^{-7} M	-12 ± 4	+34 ± 10

Other work has shown that 10^{-7}M NE is capable of stimulating cAMP production in brain tissue. These results suggest an interesting dichotomy in the effect of NE on cAMP production in olfactory tissue from rats of different ages.

542.18

MORPHOLOGICAL CHANGES IN ASTROCYTES OF AGING MICE FED NORMAL OR CALORIC RESTRICTED DIETS A.J. Castiglioni, Jr., M.E. Legare*, D.L. Busbee* and E. Tiffany-Castiglioni*. Depts. Medical Anatomy and Veterinary Anatomy, Texas A&M University, College Station, TX 77843-4458.

Dietary restriction throughout life (restriction of calories without decrease in essential nutrients) increases average and maximal lifespans in rodents (Yu, B.P., J. Gerontol. 37:130, 1982). Astrocyte hypertrophy, associated with neuronal degeneration, has been reported in aging rat hippocampus. In the present study, the effect of caloric restriction on age-related changes in the maximal diameters of astrocytes was measured. C57BL6 mice maintained on either ad libitum (NIH 31 rodent diet) or restricted (60% ad libitum with vitamin supplementation) diet, ages 0.6, 6, 19, 22, 24, and 26 months were obtained from the NIA/NCTR aging rodent facility in Jefferson, AK. Their brains were stained by a modified Golgi method and astrocyte diameters were determined with a Zeiss Videoplan image analyzer. Astroglia were significantly smaller in the frontal and parietal cerebral hemispheres of caloric-restricted mice at six months of age than those of ad libitum-fed mice. At later time points, however, no significant differences were seen between the two groups. Supported by a grant from the Alzheimer's Disease and Related Disorders Association.

542.19

SELECTIVE IMPAIRMENT OF SYMPATHOADRENAL AND CARDIOVASCULAR RESPONSES TO MU-OPIOID RECEPTOR STIMULATION DURING AGING. J.A. Kiritsy-Roy, J.B. Halter*, M. Smith*, L.C. Terry. Neuroendocrine Lab., Depts. of Neurology and Medicine and Inst. of Gerontology, Univ. of Michigan and VA Med. Center GRECC, Ann Arbor, MI 48105.

Brain regional opioid peptide concentrations and receptor densities change during aging (Agnati *et al.*, Acta Physiol Scand 128:201, 1986). To determine the potential functional significance of these changes, we examined cardiovascular and plasma catecholamine responses to a mu-selective opioid peptide, [D-Ala^2, MePhe^4, Gly(ol)^5]enkephalin (DAGO) injected intracerebroventricularly (icv) in 6 (n=11) and 24 (n=7) month old Fisher 344 rats. Guide cannulae were implanted over the lateral cerebral ventricle for icv drug injections, and catheters were placed in the carotid artery for recording systolic (SP) and diastolic (DP) blood pressures and blood sampling in conscious freely-moving rats. Blood samples (0.7 ml) were obtained before and at 5, 15 and 30 min after DAGO (1 nmole icv) for assay of plasma concentrations of norepinephrine (NE) and epinephrine (EPI).

Resting SP and DP did not differ between 6 month (SP, 118±9; DP, 95±8 mmHg) and 24 month (SP, 104±6; DP, 89±5 mmHg) old rats. Thirty min after DAGO, SP and DP increased in young rats by 25±5 and 14±4 mmHg, respectively, but not in aged rats (SP, 3±4; DP, -3±3 mmHg). The DAGO-induced increase in plasma [EPI] in 6 month old rats (21.6±4.0 nM) was blunted in 24 month old cohorts (8.2±3.5 nM, P=0.033); NE responses were similar in young (8.6±1.4 nM) and aged (6.4±1.9 nM) groups (P=0.360). Thus, there is a selective decrease in mu-opioid regulation of sympathoadrenal outflow during aging, with attenuation of EPI but not NE responses to DAGO. This indicates that NE derives from a non-adrenal source and that reduced opioid-activated sympathoadrenal outflow may account for attenuation of the pressor response to brain mu-opioid peptides in aging. (Grant support from AFAR, NIH and VA)

542.20

INCREASED Ca-INFLUX AT OLD SOLEUS NERVE TERMINALS OF THE MOUSE. W.B. Alshuaib and M.A. Fahim.Andrus Gerontology Center and Dept. of Biological Sciences, University of Southern California, Los Angeles, CA 90089-0191.

To determine whether increased transmitter release at old soleus endplates of C57BL/6J mice is caused by an altered Ca regulation, the time course of posttetanic potentiation (PTP) was used to study Ca metabolism in young (10 months) and old (24 months) mice. Evoked release was measured in 0.4 mM Ca, 2.75 mM Mg Krebs. PTP properties were studied in either 1) 0.2 mM Ca, 5.0 mM Mg Krebs; Or 2) Ca-free/EGTA Krebs to eliminate Ca influx, and thereby isolate Ca buffering. In the 0.2 mM Ca Krebs, the time constants of decay of augmentation (Ta) and potentiation (Tp) were longer in old (Ta=10.3±1.0 sec, Tp=195.3±5.4 sec) than in young (Ta=7.0±0.7 sec, Tp=78.8±6.6 sec) endplates. Quantal content (m) was positively correlated with Ta (r=0.95) and with Tp (r=0.98). In the Ca-free/EGTA Krebs, there was no difference in PTP properties between young and old terminals. These results suggest that Ca-influx is greater in old than in young terminals, this difference may underlie the increased m of old terminals.

542.21

THE EFFECTS OF AGING ON THE RAT NASAL OLFACTORY ORGAN (NOO). W.G. Lavelle and A.A. Carboni. Dept. of Surgery, Div. of Otolaryngology/Head and Neck Surgery, UMASS, Med. Center, Worcester, Ma 01655.

Without experimental intervention, degenerative lesions similar to neuritic plaques have been found in the olfactory cortical and subcortical areas of aged rats. These changes may be initiated from insult to the NOO, particularly the olfactory mucosa, by outside infectious agents or chronic exposure to environmental toxins, secondarily affecting the olfactory nerves and the bulbs.

The NOO, consisting of the olfactory mucosa, nerves and bulbs encased in bone, of 22 day and 22 mo. old rats sacrificed by vascular perfusion of aldehydes. They were decalcified, dehydrated and cut into alternating serial sections of 30 and 100um. The 30um sections were stained with hematoxylin and eosin (H&E) and the 100um sections were prepared with a slice Golgi-EM technique for electron microscopy.

Although the changes were not always bilaterally symmetrical, the nasal olfactory mucosa of the aged rats were swollen and exhibited a decrease in the number of basal, supporting and sensory cells. The neuronal perikarya seemed resistant to temporal changes. In the olfactory bulb there appeared to be a decrease in the number of olfactory nerves. The mitral cell glomerular layer exhibited a loss of synaptic density and a decrease in the size of the remaining synapses.

(Supported by BRSG #6-32724)

542.22

ELECTROPHYSIOLOGICAL ALTERATIONS IN STRIATAL NEURONS IN AGED RATS: IN VITRO STUDIES. N. Lee*, C. Cepeda*, C.S. Bailey and M.S. Levine. MRRC, UCLA, Los Angeles, CA 90024.

Our previous intracellular experiments on striatal neurons in anesthetized aged rats demonstrated decreases in evoked excitatory responses. A question that arose from that study concerned the relative contribution of presynaptic and/or postsynaptic influences to this decrease. To provide a more detailed examination of these influences, the in vitro brain slice preparation was used to measure the passive and active membrane properties of striatal neurons more accurately. Fisher 344 rats (young 3-5 months (n=8) and aged >24 months (n=10)). Striatal slices (coronal sections 400 μm thick) were prepared by standard techniques. To date we have recorded from 22 neurons in aged and 15 neurons in young rats. Average resting membrane potential (RMP), action potential (AP) amplitude and input resistance (Rin) were similar in the two age groups (-66±9.8 (mean±s.d.) vs. -65±8.2 mV RMP, 51.1±6.8 vs. 53.6±10 mV AP, 28.9±13.6 vs. 23.8±8.5 MΩ Rin for old vs. young rats respectively). Local stimulation was used to evoke excitatory postsynaptic potentials (EPSPs) and APs. Threshold currents to evoke EPSPs and APs were markedly higher in old rats (55% increase for EPSPs, 125% increase for APs). When measured at twice threshold, EPSP amplitude, duration, and latency were similar in the two groups. Many neurons in aged rats could not produce repetitive spikes during depolarizing current injections. This "accommodation" occurred in 62% (8/13 cells) in aged versus 14% (1/7 cells) in young neurons. These results indicate that there are two types of changes in aged striatum. First, there is a decrease in synaptic input upon striatal neurons such that more current is necessary to evoke responses. Second, it is difficult for striatal neurons to generate action potentials when depolarized or when activated synaptically. Supported by USPHS Grant AG 7462.

542.23

DECREASED EFFICACY OF INOSITOL 1,4,5-TRISPHOSPHATE TO ELICIT CALCIUM MOBILIZATION FROM CEREBROCORTICAL MICROSOMES OF AGED RATS. D.M. Burnett, L.C. Daniell, and N.R. Zahniser. Depts. Pharmacology, Univ. Co. Hlth Sci. Ctr., Denver, CO 80262, and Med. Col. Ga., Augusta, GA 39112.

Many processes associated with calcium homeostasis change with aging. We have examined the effects of aging on the ability of inositol 1,4,5-trisphosphate (IP_3) to mobilize calcium from microsomes prepared from cerebral cortex, hippocampus, thalamus, and cerebellum of 3, 16 and 28 month-old Fischer 344 rats. Microsomal calcium uptake and release were determined spectrofluorometrically using the fluorescent indicator Indo-1. Calcium uptake was first stimulated by the addition of ATP and then inhibited with sodium orthovanadate. No aged-related differences were found in the ability of brain microsomes to sequester calcium in response to ATP stimulation, but an interregional difference was seen. The average calcium-sequestering capacity in cerebellum was 3.4 nmol/mg protein, followed by cerebral cortex (2.6), hippocampus (2.1) and thalamus (1.4). A maximally effective dose of IP_3 (1 uM) released approximately 30% of the calcium sequestered by microsomes in all brain areas and age groups studied except in the cerebral cortex where a significant effect of aging was observed. In cortex, 1 uM IP_3 released 0.77±0.04, 0.81±0.04, and 0.40±0.02 nmol calcium/mg protein at 3, 16 and 28 months of age, respectively, corresponding to responses of 30%, 30% and 15%. Dose-response curves for IP_3 confirmed that neither the maximally effective doses (1 and 1 uM), the EC_{50} values (133 and 138 nM) nor the Hill coefficients (0.91 and 1.1) differed in cortex from 3 and 28 month-old rats. These data indicate that the efficacy of IP_3 is selectively diminished in the cerebral cortex of aged rats. Supported by USPHS AG04418 and the PMA Foundation.

542.24

ACUTE AND LONG-TERM CALORIC RESTRICTION AFFECT COORDINATED MOTOR PERFORMANCE OF THREE MOUSE GENOTYPES. M. Forster, M. Flores* and H. Lal. Department of Pharmacology, Texas College of Osteopathic Medicine, Fort Worth, TX 76107-2690.

Caloric restriction regimens which increase longevity of rodents have been shown to retard age-associated changes in CNS processes related to sensorimotor functions. The current studies investigated the effects of long-term caloric restriction on sensorimotor function in the context of different genotypes and acute diet conditions. Chronically diet-restricted or ad lib fed mice of three genotypes (C57BL/6NNia, DBA2/NNia and B6D2F$_1$) aged approximately 8, 17, or 26 months were obtained from the NCTR-NIA Project on Caloric Restriction colonies. Half of the mice in each diet condition were switched and maintained under the opposite condition for four weeks prior to an extended series behavioral and physiological tests. Restricted mice were switched to a night-feeding regimen to synchronize their circadian metabolic and behavioral variations with the ad lib mice. Age-related declines in performance capacity for a rotating-treadmill task were delayed in C57BL/6NNia and B6D2F$_1$ genotypes following long-term caloric restriction, regardless of the acute restriction condition. Acute restriction had a marked effect upon treadmill performance at each age in all genotypes, suggesting that evaluations of the effects of caloric restriction upon age-related behavioral changes will require careful consideration of the role of short-term factors associated with experimental diet conditions. [Supported by NIH grants AG07695 (H.L.) and AG06182 (M.J.F.)]

542.25

CROSS-TALK BETWEEN SECOND MESSENGER SYSTEMS AND THE REDUCTION OF AGE RELATED DEFICITS IN MUSCARINIC CONTROL OF STRIATAL DOPAMINE AUTORECEPTORS. J. A. Joseph and G. S. Roth*. Gerontology Research Center/NIA, Baltimore, MD 21224.

Possible alterations in muscarinic cholinergic (mACh) signal transduction (SnT) in senescence were studied in neostriatum (NST). ACh activation of m heteroreceptors by carbachol or oxotremorine inhibits dopamine DA autoreceptors and enhances K^+-evoked release of dopamine (EK^+ERDA) from perifused NST of 6 but not 24 mo rats. However, age deficits in EK^+ERDA are not seen if the CA^{2+} ionophore, A23187 or the putative second messenger, IP_3, are applied. Present experiments determined effects of simultaneous activation or activation/inhibition of more than one putative second messenger on EK^+ERDA (HPLC coupled to EC detection; from perifused NST with 2.5 followed by 30 mM KCl in Krebs-Ringer medium, with combinations in μM of 100 carb. to stimulate IP^3 production; 100 oxo. to inhibit cyclic-AMP production; 10 arachidonic acid, a putative sec. messenger; 25, 50 neomycin to inhibit phospholipase C and assess specificity of carb. and oxo. on IP_3) from 6 mo and 24 mo Wistar rats. Results indicated that: (a) Neomycin antagonized carb. but not oxo. EK^+ERDA. (b) EK^+ERDA to either oxo. or carb. alone was blunted in senescent NST but not to the combination. (c) EK^+ERDA to arach. alone was blunted but not to arach. -oxo. or arach.-carb. Thus, sec. mess. activation or activation inhibition reduced EK^+ERDA deficits in aged NST.

542.26

AGE RELATED CHANGES IN FAST AXONAL TRANSPORT: RETROGRADE FEEDBACK MAY REGULATE ANTEROGRADE SPEEDS. A.C. Breuer and M.B. Atkinson*. Neurology and Brain and Vascular Research, Cleveland Clinic Foundation. Cleveland, OH 44195

The hypothesis that fast axonal transport (FAT) may be involved in age-related changes in nerve and muscle has been evaluated using indirect techniques (ligation-accumulation and radiolabelling methods) with variable results. We report direct observations on the FAT of organelles in individual sciatic axons in newborn, 1,2,3 week old, 1,4, and 8 month old Sprague-Dawley rats using computer analysis of analog and digital enhanced interference contrast images recorded in real time. Mean retrograde (RETRO) FAT speeds were 1.50 ± 0.02 μm/sec and mean anterograde (ANTERO) FAT speeds were 1.47 ± 0.06 μm/sec at birth. By the end of the first month of development mean RETRO speed had *increased* significantly to 1.71 ± 0.05 μm/sec ($p<0.001$) while mean ANTERO speed had *decreased* to 1.33 ± 0.06 μm/sec (NS). Thereafter, there appears to be a convergent trend of FAT speeds at 8 months (ANTERO = 1.46 ± 0.05 and RETRO = 1.48 ± 0.02). We interpret these findings as reflecting the regulation of material delivery per unit time, that during early rapid growth, diminished RETRO feedback to the cell soma signals a high utilization rate of organelles/membrane and need for high ANTERO supply. As the animal matures and greater turn around (RETRO recycling) of membrane to the cell soma occurs, indicating less consumption, lower ANTERO supply is required.

542.27

EXOGENOUS MOUSE NERVE GROWTH FACTOR STIMULATES CHOLINE ACETYLTRANSFERASE ACTIVITY IN AGED MALE FISHER 344 RATS. L.R. Williams, K.S. Jodelis*, and M.R. Donald. CNS Diseases Research, The Upjohn Co., Kalamazoo, MI 49001.

Sensitization of basal forebrain cholinergic neurons to infusion of mouse nerve growth factor (mNGF) is axotomy-dependent in young adult animals (Brain Res., 1989). We now report that a similar activation of neurons occurs in normal, uninjured rats that are at least 2 years old. Normal 2 year old (Aged) male Fisher 344 rats had significant losses of ChAT activity compared to normal 4 month old (Young) rats in micro-dissections of the septum and diagonal band (MS/DB), and caudate-putamen (C-P),as well as the frontal, temporal, and hippocampal cortices. ChAT activity in the Young MS/DB was 2.4 pmol ACh/μg protein/min (n=10) compared to 1.9 (n=21) in the Aged MS/DB ($p<.05$). Continuous infusion of mNGF (1.2 μg/day for 2 wks) into the right lateral ventricle had little effect on ChAT activity in the basal forebrain of Young rats, as in the previous report. However, mNGF treatment induced a 150% supranormal stimulation of ChAT activity in the MS/DB and C-P (n=6, $p< 0.001$) of Aged rats. A significant stimulation of ChAT was also detected in the basal nucleus of Meynert and cortices of mNGF-treated Aged rats. This age-dependent sensitization to exogenous mNGF may underlie the behavioral improvement and morphological effects found in aged animals after NGF treatment (Nature329:65).

542.28

ALTERATION IN LEVELS OF CHOLINERGIC NEURONAL MARKERS IN ADULT AND AGED RAT BRAIN BY EXOGENOUS NERVE GROWTH FACTOR. R.J. Rylett and L.R. Williams. Dept. Physiology, Univ. of Western Ontario, London, Canada, and CNS Diseases Research, The Upjohn Co., Kalamazoo, MI 49001.

Nerve growth factor (NGF) plays a role in the survival and transmitter function of cholinergic neurons in the CNS. NGF can prevent retrograde degeneration of rat basal forebrain cholinergic neurons following axotomy (PNAS 83: 9231), and decreased levels of NGF may be related to the dysfunction associated with Alzheimer's Disease. In the present study, NGF was administered for two weeks to adult (4 month) and aged (24 month) Fisher 344 rats using an Alzet minipump. Control rats received a vehicle infusion or were untreated. Choline acetyltransferase (ChAT) and high-affinity choline uptake (HACU) activity were measured in striatum, frontal cortex, and hippocampus. In NGF-treated aged rats, HACU was significantly increased by 57% and 39% in striatum and frontal cortex, respectively, while this measure was not altered significantly in hippocampus; ChAT activity was increased by 72%, 28%, and 23% in striatum, cortex and hippocampus. In NGF-treated adult rats, HACU was increased by 86% in striatum, but not significantly changed in frontal cortex or hippocampus; ChAT activity was stimulated only in striatum. The results demonstrate for the first time that exogenous NGF can stimulate HACU in vivo, and indicate a differential sensitization of cholinergic markers in aged rats.

542.29

MORPHINE ANALGESIA AND TOLERANCE IS REDUCED IN THE AGED MOUSE. E.Quinton. Dept. of Psychology, University of Louisville, Louisville, Ky.40292.

Published data suggest that the number of opiate binding sites in the brain decreases as the organism ages. This study compared morphine (MCL) analgesia and tolerance in young(3 mos.) and old(23 mos.) C57BL/6j male mice. Groups of young and old mice were injected with 25 mg/kg MCL or saline before a hot plate (HP) or writhing (glacial acetic acid induced) test. The old saline injected mice responded more quickly on the HP but had fewer writhes than the young saline mice. The analgesic effect of MCL with the HP test was less in the old MCL group than the young, but the difference between the young and old MCL groups on the writhing test was not significant. To induce acute tolerance, a conditioning dose of 200mg/kg MCL was injected into old and young mice and 72hrs later a test dose of 25mg/kg was given, 30 min before the HP test. The young group was significantly tolerant, but the old group was not. These results suggest that old animals are differentially sensitive to painful stimuli, morphine is less effective as an analgesic, and tolerance develops less readily.

542.30

IN SITU HYBRIDIZATION ANALYSIS OF THE LOCALIZATION AND REGULATION OF SOMATOSTATIN IN AGING RAT HYPOTHALAMUS. R. M. Booze, R. Boyd*, and W. E. Sonntag*, Dept. of Physiology and Pharmacology, Bowman Gray School of Medicine, Wake Forest University, Winston-Salem, NC 27103.

Somatostatin is altered both as a consequence of normal brain aging and in Alzheimer's disease. We have previously found that somatostatin gene expression is decreased in the hypothalamus of aged rats. In these experiments, in situ hybridization was used to determine whether age-related changes in somatostatin are associated with (1) altered somatostatin gene expression in specific neuronal populations, or (2) decreased numbers of somatostatin-producing cells.

Brains from young (3-4 months), middle-aged (12-14 months), and old (22 month) male Fischer-344 rats (N=9) were frozen and cryostat-cut (16 μm). A plasmid containing the somatostatin cDNA insert in pSP65 (a gift from Dr. Marc Montminy) was used to transcribe a ^{35}S-labeled antisense RNA probe for hybridization. The cellular localization of somatostatin mRNA was evaluated following autoradiographic development. Large, densely-labeled cells were found in the paraventricular and periventricular nuclei of young rats. These same neuronal populations were readily identified in middle-aged and aged rats. However, with age the amount of label/cell decreased, with no change in cell number. These results indicate that the age-related decrease in somatostatin gene expression is a consequence of decreased expression in specific hypothalamic nuclei rather than a loss of somatostatin-producing cells. Our results suggest that alterations in somatostatin gene expression may be an early consequence of brain aging. (Supported by NIH grant AG-07752 and the American Federation for Aging Research.)

542.31

SINGLE UNITS IN THE HIPPOCAMPUS OF RATS HAVE MNEMONIC CORRELATES DURING PERFORMANCE OF A WORKING MEMORY TASK. Y. Sakurai, K. Pang, and D. Olton. Neuromnemonics Laboratory, Dept. Psychology, The Johns Hopkins University, Baltimore, MD 21218.

The role of the hippocampus in memory was examined by recording from single units in the hippocampus while rats performed a discrimination that required working memory. The task was continuous nonmatch-to-sample with two stimuli, a light and a tone. For each trial, the rat compared the current stimulus to the stimulus on the preceding trial, and responded by pressing one lever if the two stimuli were the same (match trial) or by pressing another lever if the stimuli were different (nonmatch trial). Some of these units had mnemonic correlates, i.e., changes in activity associated with a particular mnemonic demand rather than with a particular stimulus or response. For example, some units had different rates of activity in match trials and nonmatch trials, or an increased rate in a particular type of trial (i.e., light followed by tone). These patterns of activity provide information about the ways in which the hippocampus processes memory.

542.32

REDUCED BRAIN METABOLIC RESPONSE TO META-CHLOROPHENYLPIPERAZINE AFTER CHRONIC ADMINISTRATION. U.Freo*, T.T. Soncrant, D.M. Larson* and S.I. Rapoport (Spon. H. Levitan) Laboratory of Neurosciences, National Institute on Aging, NIH, Bethesda, MD 20892.

Recently we demonstrated reduced metabolic responsivity to the serotonin 1B agonist and trazodone metabolite meta-chlorophenylpiperazine (MCPP) in aged vs. young Fischer-344 rats. To evaluate the pharmacological relevance of these findings, we examined the development of metabolic tolerance to MCPP during its chronic administration. After continuous pretreatment with MCPP 2.5 mg/kg/day s.c. for 2 weeks, awake, male Fischer-344 rats aged 3 or 24 months received saline or MCPP 2.5 mg/kg as an i.p. bolus, and regional cerebral metabolic rates for glucose were measured 15 min later in 71 regions, using the quantitative, autoradiographic [^{14}C]deoxyglucose technique. Chronic pretreatment abolished hypomotility produced by acute MCPP administration in rats of both ages. Regional cerebral metabolic rates for glucose were not different in any region between age-matched MCPP-pretreated rats that received acute MCPP or saline and were similar to values in untreated rats. These results indicate that chronic treatment with MCPP abolishes behavioral and cerebral metabolic responses to acute MCPP in both 3 and 24 month old rats. Tolerance can develop in aged rats despite reduced metabolic responsivity to its acute administration.

542.33

REDUCED NUMBERS OF HYPOTHALAMIC BETA-ENDORPHIN (B-END) AND LHRH NEURONS IN AGED FEMALE C57BL/6J MICE. M.M. Miller, D.Joshi*, R.B.Billiar*, J.F. Nelson*. McGill Univiversity, Departments of Obstetrics-Gynecology and Anatomy. Montreal, Quebec, Canada, H3A 1A1.

Neuronal density of arcuate and preoptic nuclei is reduced by 30% in aged female mice (Miller et al., Neurobiol of Aging, in press), but which neurons are involved is not known. This study assessed the effects of aging on the number and distribution of LHRH and B-end-containing neurons in the rostral fore-brain. Serial coronal sections extending from caudal hypothalamus through olfactory tubercle were examined by quantitative light microscopy for number of immunoreactive neurons in young (5-6 mo) normally cycling females and in old (26-28 mo) non-cycling mice. Old animals had 17% fewer B-end-containing neurons in the arcuate nucleus ($p<.05$), and 25% fewer LHRH neurons ($p<.0001$) in the region of the preoptic area and the diagonal band of Broca compared to young mice. No change in distribution was observed for either type of neuron. The loss of B-end-containing neurons may partly account for the 30% decrease in pro-opio-melanocortin mRNA in aging mice of this strain (Nelson et al., Endocrinology, 123:340, 1988).

INFECTIOUS DISEASE

543.1

THEILER'S VIRUS ISOLATED FROM A PERSISTENTLY INFECTED OLIGODENDROGLIOMA CELL LINE FAILS TO INDUCE DEMYELINATING DISEASE IN CENTRAL NERVOUS SYSTEM OF SJL/J MICE. A. K. Patick* and M. Rodriguez* (SPON: V. Lennon) Dept. of Immunology, Mayo Clinic, Rochester, MN 55905.

Infection of the G26-20 oligodendroglioma cell line with the DA strain of Theiler's virus (DAV) results in persistent infection in which levels of infectious virus and viral antigen fluctuate in a cyclical manner. To determine if DAV isolated from this line (DAV-P) differed in pathogenicity, we inoculated susceptible strains of SJL/J mice with either wild-type(wt) DAV or DAV-P. At 45 days after infection quantitative analysis was performed by scoring each quadrant from 15-20 spinal cord coronal sections for presence or absence of inflammation and/or demyelination and expressed as a pathological score (mean $\pm$ SD). In contrast to extensive areas of demyelination and inflammation in mice infected with wt-DAV (37.5 ± 15.2), mice infected with DAV-P had no detectable demyelination or inflammation (0.0 ± 0.0). Both groups of mice mounted an immune response to DAV as indicated by presence of antibody to DAV as measured by ELISA assay against purified DAV antigen. This variant will be useful to study the viral determinants important in virus-induced demyelination. (Supported by NIH grant NS 24180, Multiple Sclerosis grant RG-1878, and Searle Scholar Award).

543.2

INTEGRIN RECEPTORS OF THE β1 SUBFAMILY REGULATE MHC CLASS II EXPRESSION IN RAT SCHWANN CELLS K.R. Jessen*, K. Bergsteinsdóttir* and R. Mirsky* (SPON: BRA) Department of Anatomy, University College London, Gower Street, London WC1E 6BT, England.

We have investigated the effect of type I collagen on the ability of rat Schwann cells (SC) to express MHC class II (Ia) molecules and the developmental regulation of this response. If SC were grown on polylysine their ability to respond to interferon-γ (IFN-γ) by class II expression was related to developmental age. Thus, 3 day INF-γ incubation of SC from embryonic day (E) 19,21, newborn or 4 day old rats resulted in class II expression on 8%, 17%, 19% and 50% of the SC, respectively. However if E-19, E-21 or newborn rat SC were grown on gelled or dried type I collagen for 2-3 days prior to incubation with IFN-γ about 50% became MHC class II positive in every case. This enhancement of class II expression was also observed with heat denatured type I collagen and when cells were grown on polylysine in medium containing soluble type I collagen, and was seen equally in medium containing serum with or without fibronectin. Antibodies (made and characterized by D. Gullberg et al, Biomedical Center, Uppsala Sweden) against the β1, chain of integrin matrix receptors blocked the effect of collagen on class II expression. These results demonstrate that type I collagen, acting via β1, type integrins, enables embryonic and early postnatal, SC to respond to IFN-γ by expressing MHC class II molecules.

543.3

HIV-1 INFECTION OF HUMAN FETAL NERVOUS SYSTEM ORGANOTYPIC CULTURES VIA AUTOLOGOUS LYMPHOCYTES. W.D. Lyman*, Y. Kress*, K.E. Tanaka*, W.C. Hatch*, K.D. Hutchins*, M. Tricoche*, W.K. Rashbaum* and R. Soeiro* (SPON: W.T. Norton). Albert Einstein Coll. Med., NY, NY 10461.

Previous studies from our laboratories have shown that HIV-1 can directly infect organotypic cultures of human fetal central nervous system (CNS) tissue. Because this may not represent the method by which HIV-1 enters and affects the CNS in vivo, studies were conducted to investigate the ability of HIV-1 to infect fetal lymphocytes and for these cells to act as a vector for HIV-1 infection of autologous organotypic CNS cultures. Fetal lymphocytes were incubated with the RF2 strain of HIV-1. Lymphocyte infection by HIV-1 was determined by nucleic acid hybridization and a syncitium-forming assay. Autologous CNS organotypic cultures were then exposed to the HIV-1 infected cells for 1, 2 or 3 weeks. Sample cultures were washed free of added HIV-1 infected lymphocytes and tested for infection by nucleic acid hybridization in combination with microscopy. Results show that CNS organotypic cultures can be infected via autologous HIV-1 infected lymphocytes and that this infection can cause pathologic changes similar to those observed in vitro using cell-free HIV-1 containing supernates. These studies may contribute to an understanding of the pathogenetic mechanisms underlying the neuropathology of AIDS. (Supported by DA 05583, DA 04583, NS 11920, AI 20671 and HL 07060.)

543.4

CHARACTERIZATION AND INFECTION OF PRIMARY HUMAN FETAL ASTROGLIAL (HFA) CELL STRAINS WITH HUMAN IMMUNODEFICIENCY VIRUS (HIV-1). J.J. CHAO and W.P. PARKS*. Depts. of Micro. & Pediatrics, Univ. of Miami Sch. of Med., Miami, FL 33136.

Human cell strains consisting predominantly of glial fibrillary acidic protein (GFAP)-positive astrocytes were established from abortuses of 8-18 weeks gestation. Several media and substrates were evaluated to determine optimal plating efficiency and growth. Media were supplemented with 5% horse serum; N5, a medium free of selected amino acids and Dulbecco's modified Eagle's medium were comparable as measured by growth rates whereas RPMI 1640 supported only limited growth. Plating efficiency was evaluated using a rat tail collagen preparation, commercial collagens, and poly-L-lysine to pretreat tissue culture flasks. A combination of rat tail collagen and poly-L-lysine treatment yielded the highest plating efficiency ($70\% \pm 5$). Under optimal conditions in the logarithmic phase of culture growth, HFA have a doubling time of 30 hours (± 6). Cell growth occurs as multilayered foci even in sparsely plated cultures. Over time, cells radiate from foci until confluence is established at cell densities of $2.5 \times 10^5/cm^2$. GFAP-positive HFA can be maintained in culture for up to 9 months with over 25 passages without obvious changes in morphology or growth characteristics. Cell surface and cytoplasmic markers were examined by immunocytochemistry and western blot analysis. Subsets of GFAP-positive HFA strains show various degrees of positivity for vimentin, MHC class II antigens, and A2B5. Neuron-specific enolase and galactose cerebroside were not detectable. Two well characterized subsets of HFA cells were infected with a cloned isolate of HIV-1 at high multiplicity of infection. Cell culture supernatants and cell lysates were monitored for infectivity by the detection of viral protein (p24) using ELISA (Coulter Immunology) at semi-weekly intervals. Thus, it is possible to establish and to define growth requirements and characteristics of *in vitro* HFA cultures for long-term viral studies with HIV-1.

543.5

MULTIPLE SCLEROSIS IN CUBANS: CORRELATION OF HIA-B8 ANTIGEN, HTLV-I ANTIBODY AND CLINICAL TYPE. W. A. Sheremata*, Violet Esquinaise, Joesph Montes, and Alan Sazant

Multiple sclerosis (MS) is diagnosed in Cuban patients referred to the Miami MS center. This population is born in the Carribean, where tropic spastic paraparesis (TSP), rather than MS, might have been predicted. We have studied a sample of 32 patients for correlations between type of illness, genetic factors and antibody to HTLV-I. 19 had relapsing-remitting MS (R/R) and 13 had progressive myelopathies (PM). Nordic features occured significantly more often with typical R/R ($p < 0.001$) than in those with a PM. When compared to 452 haplotypes in unrelated Cubans, HLA-B8 appeared to be increased in the MS population, (22% vs 10.6% of controls: NS) but most prominently in the PM population (4 X controls). Western blots were positive for HTLV-I in only 3 of 13 PM patients or 9.3% of the total. All 3 were Cubans lacking Nordic features, with myelo-pathies and possessing HLA-B8 antigen. The findings indicate a possible correlation between genetic factors, expression of demyelinating illness and antibody titers to retroviral antigens. However, TSP associated with HTLV-I infection may be mistakenly diagnosed as MS. Study of larger numbers of Cuban born patients and more sensitive antibody assays are warrranted to establish links between genetic factors and immune responses to retroviruses in this unique population.

543.6

HIV-1 expression in neural cells is dependent on non CD4 associated entry and cellular permissiveness to virus expression. Y. Mizrachi, F. Sinangil*, D.J. Volsky*. Dept. Pathol.; College of P & S, Columbia Univ.; Dept of Ped. and Med.; St Luke's/Roosevelt Hosp. Center. New York NY 10019.

Human neural cells in culture were shown to be susceptible to low producing noncytopathic self limiting infection by HIV-1. Using fluorescence dequencing (DQ) technique and viral detection methods we found that the HIV-1 viral isolates NIA, NIE and SF3 readily fuse with Neural cells, and viral core protein (p24) can be detected within the cells. Viral isolates SF33, IIIB and RF2 fused poorly. The extent of fusion of HIV-1 with neural cells was found to be similar to that occuring between HIV-1 and CD4+ lymphocytic cell lines. However, in contrast to CD4+ lymphocyte, the fusion with neural cells was CD4 independent, and could not be blocked by soluble CD4. A successful viral entry into neural cell lines culminated in a productive HIV-1 infection, except the LA-N-1 neurblastoma cell line. We have concluded that both viral neurotropism and control mechanisms of target cells limit viral production in neural cells.

543.7

INCREASED QUINOLINIC ACID AND DECREASED L-TRYPTOPHAN IN CSF AND PLASMA IN HIV INFECTION. M.P. Heyes*, B. Brew*, A. Martin*, S.P. Markey*, R. Price* and A. Salazar*. (SPON: M. Williams). NIMH, Bethesda, MD; Memorial Sloan Kettering Cancer Center, NY; Walter Reed AMC, Washington DC.

A large proportion of patients infected with HIV develop neurologic abnormalities including dementia which in severe cases is associated with infiltrates of macrophages in brain. Quinolinic acid (QUIN), an excitotoxin and L-tryptophan (L-TRP) metabolite, is increased in the CSF of AIDS patients (Heyes et al., Ann Neurol.: *In Press*) and in both plasma and brain of a mouse model of acute infection (Heyes et al., *J. Neurochem.* 51: 1946, 1988). To investigate QUIN and L-TRP metabolism and the relationship of QUIN to AIDS dementia complex (ADC), QUIN and L-TRP were quantified in CSF and plasma of 20 control and 119 HIV-infected patients. Plasma L-kynurenine (L-KYN) was measured in 9 control and 13 AIDS patients. The results (Table: *p<0.001) show markedly increased QUIN and reduced L-TRP in CSF and plasma of HIV-infected patients, particularly those with ADC and opportunistic conditions. Plasma L-KYN concentrations were also increased in AIDS.

	PLASMA			CSF	
	L-TRP (μmol/L)	L-KYN (nmol/L)	QUIN (nmol/L)	L-TRP (μmol/L)	QUIN (nmol/L)
Control	70.9±6.9	2.19±0.19	439± 54	2.27±0.01	20.6±1.1
HIV Infected	39.8±1.5*	4.68±0.60*	1570±207*	1.32±0.01*	851.3±160.1*

The results are consistent with increased L-TRP catabolism through the kynurenine pathway and may reflect activation of indoleamine-2,3-dioxygenase. In demented patients without compounding illness, significant correlations were found between log CSF QUIN and: AIDS dementia complex stage ($p<0.001$), CSF Beta 2 ($p<0.005$), serum beta 2 ($p<0.005$) and log plasma QUIN ($p<0.00001$). Azidothymidine and treatment of opportunistic conditions reduced CSF QUIN and neurologic status improved. Increased QUIN may contribute to the neurologic abnormalities of AIDS by acting as an agonist of excitatory amino acid receptors or via neurotoxicity. Strategies to reduce CSF QUIN concentrations or attenuate the excitatory effects of QUIN may prove useful in the treatment of ADC. The data indicate exploration of QUIN as a mediator of neuronal dysfunction and death in infectious disease.

543.8

DELAYED SHUNT INFECTIONS IN CHILDREN. S.J.SCHIFF and W.J.OAKES. Div. of Neurosurgery, Duke Univ. Med. Center, Durham, NC 27710.

Infections in cerebrospinal fluid shunts are most common within the first weeks following shunt insertion due to organisms implanted at surgery. We have observed unusual delayed infections occurring years or decades after insertion. Cases of shunt infection at our institution were reviewed from 1979 to 1987. Twelve cases were identified where infections occurred more than 6 months following shunt insertion, in children aged 6 mo to 17 yr. Seven had recent surgery or infections that immediately preceded shunt infection. For five patients, no antecedent surgery or infection could be identified as a presumptive source of infection, the delay to infection being 13 months to 11 years; the organisms isolated included Propionibacterium in 3 patients, 2 of which had mixed Propionibacterium and Staphylococcus epidermidis infections, alpha-hemolytic Streptococcus in 1, and in another patient no organism could be identified.

The specter of shunt infection occurring years or decades following CSF shunt implantation argues for lifetime follow-up of such patients and underscores the routine need for anaerobic culture techniques when infection is suspected. We speculate that these results may be generalizable to other patients in whom neural prostheses containing silastic are implanted.

543.9

REGULATION OF LATENT HERPES SIMPLEX VIRUS IN NEURONS *IN VITRO* C. L. Wilcox, R. L. Smith*[1], and L. I. Pizer*, Departments of Microbiology and Immunology, and [1]Pediatrics, University of Colorado Health Sciences Center, Denver, CO 80262

Herpes simplex virus (HSV) establishes a latent infection in neural crest-derived sympathetic and sensory neurons. The mechanisms regulating the establishment, maintenance, and reactivation of latent HSV remain poorly understood. To better understand herpes infection of the neuron, we have studied the virus infection in neurons *in vitro*. Previously we showed latent HSV infections are established in sympathetic neuronal cultures and that deprivation of nerve growth factor (NGF) results in reactivation of latent HSV (J. Virology 62:393-399, 1988). Recently we have investigated HSV infection in sensory neuronal cultures and found that latent HSV infection was also reactivated after NGF deprivation. To dissect the pathways involved in regulati ng reactivation of latent virus, we have utilized well-characterized pharmaclolgical agents. Recently we found that agents which elevate cyclic AMP, and that activation of protein kinase C with phorbol ester produced reactivation of latent virus. An agent, 2-aminopurine, reported to inhibit a specific class of protein kinases, prevented the reactivation of latent HSV after NGF deprivation and treatment with a cyclic-AMP analog, but did not prevent reactivation after treatment with phorbol ester. This suggests that at least two cellular pathways involving phosphorylation are likely to be important in regulating reactivation of latent HSV.

543.10

UNUSUAL PATTERN OF HERPES SIMPLEX TYPE 1 INFECTION OF NEWBORN MICE. L.S. Crnic and K.L. Vanderslice*. Departments of Pediatrics and Psychiatry University of Colorado School of Medicine, Denver, CO 80262.

We have shown that neonatal infection with an attenuated HSV-1 produces hyperactivity in mice (Crnic & Pizer, Neurotoxicol. Teratol. 10:381, 1988). How does the virus enter the CNS? Newborn mice were injected subcutaneously in the shoulder with a low virulence mutant strain of HSV-1 which lacked thymidine kinase expression. At 1,24,48,72,96,168 and 192 hrs post infection whole mice were perfused, paraffin embedded, sectioned at 7u and stained for HSV-1 using PAP immunocytochemistry. At 1 hr post injection virus had adsorbed to fat and muscle layers under the skin. By 24 hr the virus had infected these tissues, with viral antigen evident in nuclei. Virus had also reached the dorsal root ganglia and sympathetic ganglia as well as alpha motor neurons in the spinal cord. At 192 hr, destruction of brown fat and muscle was evident and virus had entered the brainstem and cerebellum. Extensive growth of HSV-1 in fat and muscle has not been reported previously. Infection of these tissues led to entry of the virus via motor as well as the usual sensory pathways to the CNS. Our previous studies of CNS infection are consistent with these routes of entry. This unusual infection is probably due to the immaturity of the animals and the unusual site of inoculation. Supported by MH00621 & MH44970.

543.11

MULTIPLE SCLEROSIS: CORRELATION OF CSF IgG SUBCLASSES TO OLIGOCLONAL BANDS. J. Losy*, P.D. Mehta* and H.M. Wisniewski*. (SPON: A. Lossinsky) Lab of Viral Immunology, NY State Inst. for Basic Rsh. in DD, Staten Island, NY 10314.

One of the most characteristic immunologic abnormalities in multiple sclerosis (MS) is occurrence of oligoclonal bands in the cerebrospinal fluid (CSF). These oligoclonal bands are defined as a number of IgG bands with restricted heterogeneity. We analyzed IgG subclasses in CSF from 10 patients with definite MS. The subclasses (IgG_1, IgG_2, IgG_3, IgG_4) were detected in unconcentrated CSF by isoelectricfocusing on agarose gel with subsequent immunoblotting and avidin-biotin alkaline phosphatase staining using mouse monoclonal antibodies to human IgG subclasses. We detected IgG_1 oligoclonal bands in all CSF samples, IgG_2 bands in 3 samples, and IgG_3 bands in 6 samples. None of the MS CSF specimens had oligoclonal bands specific to IgG_4. CSF from 3 patients with other neurologic diseases did not show oligoclonal IgG bands. The results show that oligoclonal bands in CSF consist mainly of IgG_1 and IgG_3, and these findings are similar to those seen in CSF from patients with chronic viral infections.

543.12

MORPHOLOGICAL AND BEHAVIORAL RESPONSE TO ACUTE AND SUBACUTE I.C.V. GP120 ADMINISTRATION IN THE RAT. C.B. Pert*[1] R.F. Mervis[2] J.M. Hill[1], D.E. Brenneman[3] M.R. Ruff*[1] R.C. Switzer[4] B. Warren*[5] and J.R. Glowa[5] (SPON: G.A.King)[1] NSB NIMH Bethesda, MD 20892 and Peptide Design 12321 Middlebrook Rd. Germantown, MD 20874 [2] Dept of Path and Neuropath OSU Columbus, OH 43210 [3] LDN NICHD NIH Bethesda, MD 20892 [4] U of Tenn Medical Ctr. Knoxville, TN 37920 [5] CNB NIMH Bethesda, MD 20892.

AIDS infection is frequently followed by a clinical state of cognitive impairment. The protein coat of HIV (GP120) is neurotoxic in culture (Brenneman *et al* Nature,335:6399,l988) and may be the mechanism for AIDS-related dementia. In order to study this possibility, we first administered GP120 acutely, in various concentrations, through i.c.v. cannuli in rats. After 3 wks the animals were sacrificed. Histological examination of brain tissue showed evidence of neuronal degeneration, dystrophic neurons and 'blebbing' of neurites in widespread foci. In subsequent studies rats were trained on a variant of the Morris Swim Maze. After acquisition rates were stabilized, rats were implanted with i.c.v. cannuli connected to 3 wk mini-pumps containing either GP120 or vehicle. Pumps were replaced once. GP120 appeared not to have a significant effect on performance under these conditions, suggesting that perhaps some other parameter of learning (such as the initial acquisition) may be more sensitive to its effects. Histological examination revealed far less evidence of neuronal damage with this method of GP120 administration.

543.13

CNS IgG Fc RECEPTOR HETEROGENEITY. N.S. Peress, J. Siegelman*, H.B. Fleit*, M.W. Fanger*, E. Perillo*. V.A. Medical Center, Northport NY, S.U.N.Y. at Stony Brook NY and Dartmouth Medical School, Hanover NH.

Three distinct IgG Fc receptors (FcRI, II, III) have been identified on human leukocytes with monoclonal antibodies. We have used these mAb to immunohistochemically characterize the human brain FcR which have been described on mononuclear cells in choroid plexus, periventricular tissues and leptomeninges. MAb 32 reactivity (FcRI) was only occasionally present in the CNS where it was seen most often in the choroid plexus. In contrast, mAb IV.3 reactivity (FcRII) was consistently present in leptomeninges, including brain perivascular regions, arachnoid granulations and choroid plexus stroma. Some samples of subependymal periventricular tissue also displayed FcRII. MAb 3G8 reactivity (FcRIII) was only seen in the subependymal periventricular tissues. These results demonstrate that regions of adult brain which produce CSF and border on CSF and vascular compartments contain mononuclear cells with the spectrum of FcR heterogeneity seen in systemic monocytes and macrophages. The distribution of these cells in brain suggests that they have a protective barrier function which may however be turned against the host in certain immune mediated disorders, in particular Multiple Sclerosis a disease characterized by macrophage mediated periventricular, perivascular and subpial areas of demyelination.

SYNAPTOGENESIS II

544.1

PHYSIOLOGICAL DEVELOPMENT OF THE DROSOPHILA GIANT FIBER PATHWAY DURING METAMORPHOSIS. E. Parks* and W. J. Costello. Dept. of Zool./Biomed. Sci. & Coll. Osteopathic Med., Ohio University, Athens, OH 45701.

The giant fiber (GF) pathway of Drosophila melanogaster mediates the stereotyped escape response of the adult. The GF drives, via electrical synapses, (1) the jump muscle motoneuron (TTMn) which activates the jump muscle (TTM) and (2) the interneuron, PSI,which chemically synapses onto the 5 wing depressor motoneurons (DLMn) which in turn activate the wing depressor muscle (DLM). The timing of the physiological connectivity of these GF pathway components during metamorphosis was established in wild-type flies (Canton-S). White prepupa=0h; eclosion occurs @ ~96-100h (25°C).

Brain and thoracic stimulation (@22°C) were used to separate central connectivity from peripheral connectivity. Brain stimulation activated the entire GF circuit; thoracic stimulation directly activated the motor axons of TTMn and the DLMn's in the peripheral nerve PDMN.The entire central GF pathway functioned by 86h. The peripheral neuromuscular synapses developed earlier. DLMn synapses were active by 76h; TTMn synapses matured slightly later (~78-80h). The latency of the GF pathway gradually decreased with maturity. Latencies with brain stimulation @ 86h were 2.32ms for DLM and 1.35ms for TTM. Newly eclosed flies had a DLM latency of 1.38ms and a TTM latency of 0.79ms with GF stimulation. Calculations show that most of the latency difference is apparently due to an increase in GF conduction velocity; GF diameter increases almost twofold from 66h to eclosion.

544.2

DYNAMICS OF FREQUENCY AND SIZE CHANGES IN THE PHOTORECEPTOR SYNAPSES OF THE LAMINA OF THE FLY'S OPTIC LOBE. J. Rybak* and I.A. Meinertzhagen. (SPON: A. Fröhlich). Life Sciences Centre, Dalhousie University, Halifax, NS, Canada B3H 4J1.

To examine the extent and dynamics of its turnover, the sizes and frequencies of an identified class of synaptic contacts formed by terminals of photoreceptors were characterized using quantitative EM methods. The influence of visual experience upon these parameters was examined in the first optic neuropile, or lamina, of the fly Musca following either rearing in constant dark or light, or following recent reversals between the two. Synaptic frequencies were higher, by 10-15%, following light-rearing, but both decrease during the first 24hrs. The size of synaptic contacts changes as a function of different visual experience, but these changes differ at different ages. During the first 24hrs, light decreases mean synaptic size through the appearance of extra small synapses. These size distributions alter most clearly with reversals in visual experience; for example, the effect of 6hrs light following 24hrs dark-rearing is to increase the number of synapses >0.22μm over those found in 30-hr dark-reared flies. In addition, a more rapid effect of light following darkness is to cause the appearance of some small synapses. These differences in the distribution of synaptic sizes may be interpreted as a result either of the growth of individual synapses and/or of their generation and loss, i.e. their turnover.

Supported by NIH grant EY 03592 (I.A.M.) and DAAD (J.R.).

544.3

SYNAPTIC DISASSEMBLY AFTER PHOTO-DEGENERATION OF RECEPTOR TERMINALS IN THE LAMINA OF THE FLY'S OPTIC LOBE. J.H. Brand-stätter* S.R. Shaw and I.A. Meinertzhagen. Life Sciences Centre, Dalhousie University, Halifax, N.S., Canada B3H 4J1.

A recent technique for photo-degeneration of receptor cells in the compound eye (Picaud, S. et al)., Neurosci. Lett., 95:24, 1988) offers the opportunity to examine,after a precise onset, the time course of changes at an identified population of afferent synapses in the fly Musca. Following intraretinal injection of ca 25nl 0.5% sulfarhodamine 101, the eye is illuminated with an epifluorescence spot of 560nm light. The eye is processed for EM, and individual cartridges, the modules of lamina neuropile, are examined in cross section. Degeneration amongst terminals is asynchronous but progressive. Receptor terminals shrink, their cytoplasm darkens and mitochondria show characteristic changes. Synaptic sites disappear in the following sequence: 1) the T-shaped presynaptic ribbon in the degenerating terminal is lost; 2) the postsynaptic ensemble separates from the terminal, with postsynaptic cisternae in L1 and L2 still distinguishable; 3) the processes of a glial cell insinuate between pre- and postsynaptic membranes; and 4) the components of the ensemble then separate. As a result, the frequencies of presynaptic ribbons [or of postsynaptic sites] decline < 37%[22%] by 1hr; < 84% [62%] by 8hr; and < 100% [73%] by 24hr. Early on, synaptic frequencies fall in proportion with terminal perimeter shrinkage, so that synaptic spacing densities are conserved until the final stage of degeneration.

Supported by grants EY 03592 (IAM) and NSERC A 9593 (SRS).

544.4

unc-104, A GENE REQUIRED FOR FORMATION OF CHEMICAL SYNAPSES IN THE NEMATODE. D.H. Hall, A. Jeyaprakash*, A.J. Otsuka* and E.M. Hedgecock*.Dept. Neurosci., AECOM, Bronx, NY 10461; Dept. Genetics, University of California, Berkeley, CA 94720; Dept. Biology, Johns Hopkins U., Baltimore, MD 21218.

Mutations of the unc-104 gene in the nematode Caenorhabditis elegans have been isolated both by chemical and transposon induced mutagenesis. Some homozygous viable strains are paralysed, while others are merely uncoordinated. A putative null allele which arrests in the first larval stage is currently under morphological study. Two viable paralysed strains have been studied in thin sections through the adult nerve ring and ventral cord, and compared to wild type. In both strains, neurons differentiate to form proper nerve cords and a nerve ring. However, many neurons have excess synaptic vesicles in their somata and all axons are reduced in caliber and lacking in vesicles. Both chemical synaptic contacts and neuromuscular junctions are greatly reduced in number: less than 50% of normal in unc-104 e1265 and less than 20% of normal in unc-104 rh43. This reduction is marked by a general loss of presynaptic densities. Gap junctions are still present between neurons and between muscles in apparently normal fashion.

The unc-104 gene has recently been cloned by transposon tagging as overlapping cDNA fragments and sequenced. The open reading frame deduced from these DNA fragments is not strongly homologous to sequences in the Genbank or PIR data bases. The 5.6 kb mRNA message seen on Northern blots could code for a 1860 amino acid protein. The unc-104 gene product may have a role in synapse formation or axonal transport.

544.5

ONSET OF SYNAPTOGENESIS BY SUPRASPINAL NEURONS IN THE CHICK EMBRYO SPINAL CORD. T. Shiga* and R. Oppenheim (SPON: W. K. O'Steen). Dept. of Anatomy, Wake Forest University School of Medicine, Winston-Salem, NC 27103.

Following the injection of horseradish peroxidase (HRP) into the brachial spinal cord of the chick on embryonic day (E) 4.5 retrogradely labelled neurons are found in the brainstem (Okado & Oppenheim, J. Comp. Neurol., 1985, 232:143). By contrast, following high cervical spinal transection, functional (behavioral) deficits are not observed until E10 (Oppenheim, J. Comp. Neurol., 1975, 160:37). To determine whether this temporal difference between projections and function reflects a delay in synaptogenesis, we looked for the presence of anterogradely HRP-labelled pre-synaptic terminals in brachial cord following injections of HRP into the brainstem at ages from E3.5 to E7. The first HRP-labelled synapses were found in the ventral and lateral marginal zones on E6 and were axo-dendritic in nature. Although some axo-dendritic synapses were observed in the brachial cord prior to E6 these were always unlabelled and probably arise from propriospinal sources (Oppenheim et al., J. Comp. Neurol., 1988, 275:159). We conclude that there is a delay of about 2 days between the arrival of supraspinal fibers and synapse formation in the brachial cord. Electrophysiological techniques may be needed to determine the onset of transmission in these early synapses.

Supported by NSF 8707290.

544.6

INTRASPINAL SYNATOGENESIS OF CGRP PRIMARY AFFERENTS INDUCED BY NERVE GROWTH FACTOR DEPRIVATION, C. E. Hulsebosch and S. M. Carlton, Marine Biomed. Inst. and Dept. of Anat. and Neurosci., The Univ. of Texas Med. Br., Galv., TX. 77550.

Previous studies indicate that Nerve Growth Factor (NGF) is an important neurotrophic source for primary afferent neurons in vivo. Daily injections of antibodies to NGF (ANTI-NGF) to rats from birth for one month provide a molecular denervation which induces intraspinal sprouting of unmyelinated primary afferent fibers in Lissauer's tract and the dorsal horn as determined by ultrastructural fiber counts and increased Calcitonin gene-related peptide (CGRP) immunoreactivity in adult ANTI-NGF rats compared to same age untreated littermates. The increased CGRP dorsal horn immunoreactivity is hypothesized to represent an increase in terminal number. Other possible mechanisms such as terminal swelling allowing more reactive antigen and/or upregulation of CGRP without concomitant terminal number increase could account for the previous observations. To begin to test these possibilities, the mean tangent diameter (D) of CGRP terminals measured in ANTI-NGF treated rats (n=38) was $0.86 \pm .25$ μm compared to untreated same age littermates (n=30), $0.84 \pm .23$ μm, which is not statistically different. This data indicates that no terminal swelling occurred as a result of ANTI-NGF treatment. Accordingly, to test the hypothesis of CGRP terminal number increase, these data will be incorporated into a stereological analysis of the dorsal horn in ANTI-NGF and untreated rats.

544.7

DEVELOPMENTAL CHANGES IN NEUROTRANSMITTER RECEPTORS ON IDENTIFIED RAT MOTONEURONS IN VITRO. P.A. St. John, Dept. of Anatomy and Prog. for Neurosci., Univ. of Arizona, Tucson, AZ 85724.

Much is known about changes in acetylcholine receptors during synaptogenesis at the neuromuscular junction. Much less is known about changes in other types of receptors during synaptogenesis at neuron-to-neuron synapses. In an effort to obtain such information, experiments are being performed to measure changes in the numbers and subcellular distributions of neurotransmitter receptors on rat spinal motoneurons that have been specifically labeled and grown in culture, either in isolation from other neurons or in co-culture with other neurons that can form synapses onto them. Motoneurons are labeled in fetal rats at embryonic day 14 by injection of the fluorescent carbocyanine dye di-I throughout the limb muscles, as previously described for other tracers (Schaffner, St. John, & Barker; J. Neurosci. 7:3088), with 18-24 hr allowed for retrograde transport. Dissociated cell suspensions from the spinal cords of these animals are either plated directly to form "mixed" cultures in which the motoneurons carry the di-I label, or passed through a fluorescence-activated cell sorter to separate the motoneurons from other cells. Radioactive and fluorescent ligands for substance P (SP) receptors are being used to detect changes in the number and subcellular distribution of these receptors on the motoneurons before, during, and after formation of synapses on them in vitro. We have synthesized a fluorescent derivative of SP using the method of Payan et al. (J. Clin. Invest. 74:1532). Initial results with this probe indicate that motoneurons have no detectable SP receptors at the time of dissociation, although they do express such receptors later in development. Experiments are in progress to examine the possible role of synaptogenesis in receptor expression in vitro. Supported by NSF (BNS-8808506) and the Arizona Disease Control Research Comm.

544.8

SYNAPTOGENESIS IN HIPPOCAMPAL CULTURES: AXONS AND DENDRITES BECOME COMPETENT TO FORM SYNAPSES AT DIFFERENT STAGES OF DEVELOPMENT. T.L. Fletcher[1], P. De Camilli[2] and G.A. Banker[1] [1]Dept. of Anatomy, Albany Medical College, Albany, NY 12208; [2]Dept. of Cell Biology, Yale University School of Medicine, New Haven, CT 06510.

Hippocampal neurons in culture develop extensive axonal and dendritic arbors and form numerous synapses with one another. We have previously shown that immunofluorescence localization of Synapsin I can be used to identify presynaptic specializations in these cultures. Synapses first appear on day 3 or 4 in culture, and their number increases rapidly thereafter. Synapses were never observed before day 3, even though numerous contacts between axons and other cells developed within the first 24 hrs in culture.

The delay in the appearance of presynaptic specializations could be related to maturational events in the presynaptic axon or in the postsynaptic target. To distinguish between these two possibilities, newly dissociated neurons were added to cultures containing mature neurons at very low density. After only one day of co-culture, there was a 5-fold increase in the number of synapses along the cell bodies and dendrites of the mature neurons, compared to mature neurons cultured alone at a comparably low density. In contrast, when the axons of mature cells contacted immature cells, synapses were first observed only after co-culture for 3 days. These results suggest that the axons of hippocampal neurons have the capacity to form presynaptic specializations containing clusters of synaptic vesicles soon after they emerge, provided they encounter appropriate targets. On the other hand, the cell bodies and dendrites of hippocampal neurons are not capable of inducing the formation of presynaptic specializations until they have matured for at least 3 days in culture.

544.9

ULTRASTRUCTURE OF THE DEVELOPING CHICK TANGENTIAL VESTIBULAR NUCLEUS FOLLOWING OTOCYST ABLATION. R.S. Petralia, S.S. Gill*, and K.D. Peusner. George Washington Univ. Sch. of Med., Washington, D.C. 20037.

The tangential nucleus (TN) is a primary vestibular nucleus whose neurons migrate and begin to differentiate between 5 and 8 embryonic days. Following early otocyst ablation, which prevents the ingrowth of primary vestibular afferents, TN neurons still migrate and differentiate normally up to 8 days (Golgi studies; Peusner and Morest 1977). Ultrastructural studies of normal embryos from 5 to 8 days have shown that synapses in the TN are formed mainly by longitudinal fibers of unknown origins on the processes of primitive epithelial cells (PEC). PEC are neuron precursors.

In the present ultrastructural study of otocyst-ablated embryos, we have determined that synapses form at the normal times (between 5 and 8 days) and between the normal synaptic partners. Therefore, migration and differentiation of TN neurons may depend in part on synapse formation by the longitudinal fibers, some of which must be of non-vestibular origins. Supported by NIH grant R01 NS18108.

544.10

DEVELOPMENT OF TWO TYPES OF CENTRIFUGAL FIBERS IN THE CHICK RETINA: A STUDY WITH DiI IN FIXED TISSUE. B. Fritzsch, P.G.H. Clarke* and M.-D. Crapon de Caprona* (SPON. M.J.Reymond) Dept. of Anatomy, Univ. Lausanne, CH-1005 Switzerland .

We have studied the development of fibers reaching the inner plexiform layer (IPL), by applying DiI on the cut optic nerve of formalin fixed chicks and embryos between 10 days of incubation (E10) and 5 days after hatching, thereby labeling centrifugal fibers as well as ganglion cells and their processes. Processes that arose directly from the optic nerve head were considered to be centrifugal fibers. Shortly after these fibers reach deep into the IPL (E13), two types can be identified; one has numerous branches and the other not. At E15 the initially minor differences between the two fiber types have become more pronounced, one type showing a single and very restricted terminal area, whereas the other consists of multiple branches each of which courses for several hundred microns in the terminal layer. At E18 and in hatched chicks the restricted type terminates on flask-shaped cells in the amacrine sublayer, whereas the widespread type remains in lamina 1 of the IPL, spreading tangentially for more than 1mm. The differences in size and pattern reported here are much more pronounced than in most previous reports, but match the description of Dogiel (1895). We have evidence that the restricted type originates from neurons of the isthmo-optic nucleus and the widespread type from ectopic isthmo-optic cells.

544.11

TIME OF ORIGIN OF TARGET-SPECIFIC SYMPATHETIC GANGLION NEURONS. L.L. Wright, A.F. Elshaar, and C. Skelton, Dept. Anatomy, Boston Univ. Sch. Med., Boston, MA 02118.

Much of what we know about postganglionic sympathetic neurons is based on studies of whole ganglia. However, neurons of the rat superior cervical ganglion (SCG) innervate a wide variety of tissues, including the submandibular gland and iris, and are therefore potentially functionally heterogeneous. To determine whether neurons born at different times selectively innervate different target tissues, pregnant rats were injected with tritiated thymidine on gestation day (G)15, 18, or 20 to label neurons of the fetuses undergoing their final mitotic divisions at these ages. To identify target-specific subpopulations of SCG neurons, pups were injected bilaterally with 1% fluorogold in the submandibular gland (5 ul) or in the anterior chamber of the eye (1 ul), three days prior to sacrifice at postnatal day (P)15. Superior cervical ganglia (SCGs) were then removed and processed for autoradiography .

There was no gender difference in the overall percentage of total SCG neurons born on G15-16 (6.8% in males, 7.7% in females). However, a sex X target ANOVA revealed a significant target effect and sex X target interactions. In females, 20% of the SMG-projecting neurons labeled on P12 were born on G15-16, while none of the eye-projecting neurons were born on this day. In males, 2.4% of the SMG-projecting neurons were born on G15-16, and 10% of the eye-projecting neurons were born on this day. These data support the hypothesis that the choice of peripheral target tissue innervated is related to the time of final division of SCG neurons.

Supported in part by NIH grant NS21577.

544.12

ACQUISITION OF SYNAPTIC PROPERTIES BY THE GROWTH CONES OF SEROTONERGIC AXONS IN DEVELOPING RAT BRAIN. N. Ivgy-May*, H. Tamir and M.D. Gershon, (SPON: L. Role) Dept. Anat. and Cell Biol. Columbia Univ. P&S, New York, NY. 10032.

Stages in the remodelling of the growth cones of serotonergic axons into synapses were investigated. Properties that were examined included the acquisition of synaptic vesicles and the plasma membrane 5-HT transporter. We have previously reported that 5-HT is present and that high affinity ^{3}H-imipramine binding sites are significantly enriched in growth cones isolated at day E15 from developing rat brain. We now report that isolated growth cones (IGC) take up ^{3}H-5-HT when incubated with that amine (0.5μM). This uptake is inhibited by fluoxetine (20 μM) and is temperature dependent. The specific uptake of ^{3}H-5-HT develops prior to E20; therefore the ^{3}H-imipramine binding sites in IGC are probably associated with the 5-HT transporter. The ability of reserpine to deplete the 5-HT and the presence of serotonin binding protein (SBP) were used as a markers for the presence of 5-HT-storing synaptic vesicles. Treatment of dams with reserpine (5 mg/kg) does not affect the 5-HT concentration in IGC at E15, but depletes 5-HT from IGC at E20. Administration of reserpine to postnatal animals also depletes 5-HT from IGC. Immunoblots showed that 45 kDa SBP is present and enriched in IGC at E20. These observations suggest that the growth cones of serotonergic axons acquire the plasma membrane 5-HT transporter before they contain synaptic vesicles; however, synaptic vesicles appear in 5-HT-containing growth cones and thus are present prior to the formation of synapses. The early development of synaptic mechanisms in growth cones is consistent with the possibility that release of 5-HT from growing axons may play a role in early development, even before serotonergic synapses are formed. Supported by grants MH 37575 , NS 15547, and NS 07062.

544.13

SYNAPTOPHYSIN-LIKE IMMUNOREACTIVITY IN THE DENTATE GYRUS OF THE RAT: RESPONSE TO PERFORANT PATH TRANSSECTION. A.M. Fagan, E. Masliah*, R.D. Terry* and F.H. Gage. Dept. of Neurosciences, University of California, San Diego, La Jolla, CA 92093.

Synaptophysin is a synaptic vesicle-associated protein which has been used to study synaptic populations in the nervous system. A monoclonal antibody against synaptophysin (SY-38) has been shown to label presynaptic terminals, giving a punctate staining pattern (Wiedenmann and Franke, 1985). The dentate gyrus provides a model system in which to investigate changes in synaptic organization after damage. Immunostaining in frozen sections of normal rat brain reveals a laminated pattern in the dentate gyrus consistent with the known synaptic density. Four days following unilateral transsection of the perforant path we observed a decrease in synaptophysin immunoreactivity in the denervated outer molecular layer. Further studies are in progress to investigate the characteristics, time course and quantification of these changes.

544.14

INCREASES IN mRNA FOR CYTOSKELETAL PROTEINS WITHIN THE DENERVATED NEUROPIL OF THE DENTATE GYRUS. L. L. Phillips and O. Steward. Div. of Neurosurgery, Medical College of Virginia, Richmond, VA 23298 and Dept. of Neuroscience, University of Virginia Sch. of Med., Charlottesville, VA 22908.

Following destruction of entorhinal cortical (EC) input to the rat dentate gyrus (DG) there is an increase in the incorporation of radiolabeled amino acids into protein within the denervated dendritic laminae (Phillips, et al., Mol. Br. Res. 2:257,1987). The present study utilized in situ hybridization to determine whether there was an increase in the messenger RNAs (mRNAs) for b-actin and b-tubulin within the dentate neuropil during the time periods of increased protein synthesis. At 2-21 days after a unilateral EC lesion, brain sections were hybridized with 3H-riboprobes for chick b-actin or chick b-tubulin mRNA. Light autoradiograms showed an increase in mRNA for b-actin within the denervated neuropil that peaked at 4 days postlesion when compared to the intact contralateral DG. A similar increase was observed using the riboprobe for b-tubulin mRNA, except that the peak in neuropil radiolabeling was at 6-8 days postlesion. Cell body grain density was not different between sides for b-actin and b-tubulin mRNA. These results suggest that local protein synthesis within the denervated neuropil of the rat DG involves, in part, an increase in the production of the cytoskeletal polypeptides actin and tubulin. Supported by NS 27225 to L.P. and NS 12333 to O.S.

544.15

EFFECTS OF DIFFERENTIALLY-TIMED INJECTIONS OF METHYLAZOXYMETHANOL ACETATE (MAM) ON THE ANATOMY AND NEUROCHEMISTRY OF THE HIPPOCAMPUS AND THE HABENULO-INTERPEDUNCULAR SYSTEM

A.Contestabile*,M. Virgili* and O. Barnabei* (SPON:European Neuroscience Association). Departments of Biology, Universities of Catania and Bologna,ITALY.

The use of gestational injections of MAM has been proposed by Coyle and co-workers as an useful model in which to study the alterations of neuronal circuitry and of the balance between different neurotransmitter systems. It has also been suggested that this experimental model may be used for better understanding basic mechanisms of quantitative matching between neuronal populations and their synaptic connections. In the habenulo-interpeduncular system,MAM injections have been timed in order to obtain a small or a large reduction in the size of the medial habenula,while the interpeduncular nucleus is either slightly or not affected and alterations of a cholinergic marker have been measured. In the hippocampus,differentially-timed gestational injections result in small or large numerical decrease of the pyramidal neurons while the granule cells of the fascia dentata are essentially spared. Cholinergic,GABAergic and glutamatergic markers have been measured in the hippocampus.

544.16

NMDA ACTIVATION AND SYNAPTIC PLASTICITY.

T.L. Petit. W.J. Brooks*. R. Lo*. and J.C. LeBoutillier*. Dept. of Psychology, Univ. of Toronto. Scarborough. Ont., Canada M1C 1A4

Learning and memory are thought to depend on synaptic plasticity induced by NMDA receptor activation. Developmental synaptogenesis is a model of learning and memory. If NMDA mediates synaptogenesis, NMDA administration during development should induce increased synaptogenesis but sensitivity to NMDA should decline with age. Rat pups of different developmental ages were given intraperatoneal injections of NMDA. The rat pups were sacrificed after various brief time periods, cortical sections dissected and processed for electron microscopy. Photographic analyses of synapses within the molecular layer of cerebral cortex revealed a rapid NMDA mediated increase in synaptic number. This ability of NMDA to rapidly induce synaptogenesis declined over the course of development.

544.17

NMDA ANTAGONISTS AND SYNAPTIC DEVELOPMENT.

W.J. Brooks*. J.C. LeBoutillier*. R. Lo*. and T.L. Petit (SPON: B.S. Scott) Dept. of Psychology, Univ. of Toronto. Scarborough. Ont., Canada M1C 1A4

Synaptic plasticity induced by neuronal activation is thought to provide a physiological basis for learning and memory, and be dependent on NMDA receptor activation. Developmental synaptogenesis is a model of learning and memory.

The possible involvement of the NMDA receptor in developmental synaptogenesis was investigated using the non-competitive NMDA antagonist, phencyclidine (PCP). Five day old rat pups were administered daily subcutaneous injections of 10.0 mg/kg PCP for two weeks (the period of maximal neocortical synaptogenesis). The pups were sacrificed (P20), and cortical sections processed for electron microscopy. Analyses revealed a 24% decrease in the number of synapses within the molecular layer of cerebral cortex in rats administered PCP. This finding suggests that developmental synaptogenesis is dependent on NMDA receptor activation.

544.18

SYNAPTIC PLASTICITY DURING REACTIVE SYNAPTOGENESIS.

J.C. LeBoutillier*, W.J. Brooks*, D. Anthes* and T.L. Petit (SPON: J.W. Gurd). Dept. of Psychology. Univ. of Toronto, Ontario, Canada, M1C 1A4.

Lesions of the entorhinal cortex result in synaptogenesis in the dentate gyrus. Adult reactive synaptogenesis represents a possible model for studying the synaptic plasticity underlying learning and memory.

Young adult rats were administered unilateral lesions of the entorhinal cortex and sacrificed after 3, 6, 10, 15 and 30 days. Hippocampal sections were dissected out and processed for electron microscopy. Analysis revealed synaptic decline followed by synaptogenesis. Several synaptic features showed a time dependent change reminiscent of the maturation of newly formed synapses during development. These results suggest that adult synaptogenesis and maturation parallel developmental events. Some changes were also seen in synapses previously considered "control hippocampal areas".

544.19

CHARACTERIZATION OF THE SNAP-25 PROTEIN AND mRNA IN DEVELOPING AND ADULT BRAIN.

George A. Oyler*, J.W. Polli*, M.C. Wilson+, and M.L.Billingsley. (SPON: R. Lehman) Dept. Pharmacology, Penn State Univ. College of Medicine , Hershey, PA 17033 and Scripps Clinic, La Jolla , CA 92037.

SNAP-25 (Synaptosomal Associated Protein, 25 KDa) is a developmentally regulated protein present in presynaptic terminals of specific regions in mammalian and avian CNS. Expression and localization of SNAP-25 mRNA and protein during development of rat CNS were explored using Northern blotting, in situ hybridization, immunoblotting, and immunocytochemistry. Both mRNA and protein levels increased significantly from E15 to adult as detected by Northern and Western blots. On E15, two immunoreactive SNAP-25 peptides (25 and 27 KDa) were detected in synaptosomes; the 27 KDa peptide was absent by PND 5. Immunocytochemistry revealed that the SNAP-25 protein was enriched in axons of rat hippocampal fimbria and corticospinal tracts during PND 1-14. In adult animals, immunoreactivity was confined to presynaptic terminals of mossy fibers, lateral septal nuclei, and neocortex, but was absent from fimbrial and corticospinal fibers. Subcellular fractionation indicated that SNAP-25 was tightly associated with synaptosomal membranes; the protein was not remived by 1 M NaCl, but was solubilized by 1% Triton X-100. The differential localization of SNAP-25 in axons during development and presynaptic terminals in adult brain may suggest a role for SNAP-25 in plasticity of the developing axon and adult synapse. Supported by grants from NIH (MLB and MCW), PMA Foundation (JWP), and March of Dimes (GAO).

544.20

IMMUNOCYTOCHEMICAL LOCALIZATION OF THE GROWTH-ASSOCIATED ANTIGEN 5B4, IN DEVELOPING AND ADULT RAT BRAIN.

A.A. Alcantara, K.H. Pfenninger and W.T. Greenough. Depts. Psych. & Cell and Struct. Bio., Beckman Inst., Univ. IL, Champaign 61820 & Univ. CO Hlth Sci. Ctr., Denver 80262.

The 5B4 antigen, a member of the N-CAM family identified in neural growth cones, has been implicated in neuronal growth and synaptogenesis (Ellis et al. J. Cell Biol 101:1977, 1985). The distribution pattern we have identified with light and electron microscopy provides additional evidence for a possible role in synaptogenesis in the CNS. Using an immunoperoxidase method with the monoclonal antibody Anti-5B4, we identified antigenic sites in cryosections of both developing (P0-P31) and young adult rat brain. The greatest immunoreactivity in developing animals corresponded to regions undergoing process extension or migration, such as the cortical plate or upper layers of cerebral cortex, developing fiber tract regions and the internal granule layer of cerebellum. The punctate pattern of staining in adult animals was greatest in neuropil regions of the brain, including superficial layers of cerebral cortex and molecular layers of hippocampus and cerebellum. Fiber tracts and some subcortical areas had very low staining while other subcortical neuropil regions stained. Initial cerebellum EM studies show 5B4 immunoreactivity corresponding to the LM pattern, including parallel fibers in adults and growth cones at 10 days. Support: 1 T3 MH-18882 & MH-35321.

544.21

DEVELOPMENTAL EXPRESSION AND EVOLUTIONARY CONSERVATION IDENTIFY A MAJOR SYNAPTOSOMAL PROTEIN.
S. Catsicas, D. Larhammar #, A. Blomqvist*#, R.J. Milner, and M.C. Wilson, Research Institute of Scripps Clinic , La Jolla CA-92037, USA; and #Department of Medical Genetics, Uppsala University, S-751 23 Uppsala, Sweden.

First described in the mouse, Synaptosomal Associated Protein-25 (SNAP-25), is a cytoplasmic protein mainly found in axons and terminals, and is thought to play a role in synapse function via its association with vesicles and presynaptic membranes (Oyler et al. submitted). Chick cDNA clones encoding SNAP-25 were obtained by screening a cDNA library from embryonic day 15 chick retinas, with a probe to the coding region of the mouse SNAP-25. The sequences of the chick cDNAs show a surprisingly high conservation with the mouse cDNA within the open reading frame, with 92% nucleotide and 100% amino acid sequence identity. Given this identity, we have used existing antisera, raised to the carboxyl 12 amino acids of the SNAP-25 peptide sequence in mouse, for our study in the chick. Motor and spinal ganglia neurons of the spinal cord, as well as amacrine and ganglion cells of the retina, express high levels of SNAP-25. Expression begins late during neuronal differentiation, when neurons establish contacts with their targets, and make synapses.

The evolutionary conservation, and the temporal expression of SNAP-25, lead us to propose that it may play a key role in synapse function. Also, the developmental expression of the SNAP-25 gene suggests that it may be one of a series of "synapse specific" genes regulated by interactions between growing axons and their targets.

544.22

Laminar distribution of Na,K-ATPase in the developing chick retina. H-C. T. Tsui, C.S.Kim* and W.L.Klein, Dept. Neurobiology and Physiology, Northwestern University, Evanston, IL 60208

Sodium potassium ATPase (Na,K-ATPase) activity affects membrane potential and intracellular calcium concentration, which are important factors in controlling transmitter release at the mature synapse and in controlling neurite outgrowth before synapse formation. In this study we have examined the distribution of Na,K-ATPase in the chick retina at the time of neurite outgrowth and synapse formation. Immunofluorescence experiments of cultured cells showed that a subpopulation of the neurite-bearing cells was strongly labeled. Labeled cells had a relatively homogeneous staining pattern on cell bodies, neurites and growth cones. In contrast to the uniform labeling on individual cells in culture conditions, heterogeneous labeling was found in intact embryonic retina. Distinct bands of immunoreactivity were seen in the inner plexiform layer in retinas older than E11. EM examination that individual processes were labeled strongly only within the discrete stained bands, but not outside the stained bands, suggesting distinct parts of neurites were enriched with Na,K-ATPase. The laminar pattern at the plexiform layer suggest that Na,K-ATPase was enriched at potential synaptic layer. The absence of this kind of local concentration of ATPase in cultured conditions suggests that this accumulation may depend on correct cell-cell interaction.

SUBCORTICAL VISUAL PATHWAYS IV

545.1

CONDUCTION VELOCITIES OF RETINOFUGAL FIBERS INCREASE BETWEEN OPTIC NERVE AND TRACT IN FERRETS. G.E. Baker* and M.P. Stryker, Dept. of Human Anatomy, Univ. of Oxford, Oxford OX1 3QX, U.K. and Dept. of Physiology, Univ. of California, San Francisco, CA 94143-0444.

The largest retinal ganglion cell axons in the ferret increase in caliber as they course centrally from optic nerve to tract (*Soc. Neurosci. Abstr. 14*: 992, 1988). Since axon diameter is proportional to conduction velocity, we were able to confirm and extend this finding by measuring the conduction velocities of the two largest classes of retinofugal fibers from records of the compound action potential.

Conduction times for the shortest latency negative peak (t_1), thought to represent the axons of Y or α retinal ganglion cells, and for the slower peak (t_2), thought to represent X or β-cell axons, were measured in 5 adult sable ferrets. Electrodes were positioned near the optic disc, in the optic nerve just behind the eye, in the prechiasmatic nerve, in the optic chiasm, and in the pregeniculate part of the optic tract. Conduction distances were measured directly with silk thread or computed from reconstructed serial sections.

Conduction velocities for the t_1 peak in the tract (40 ± 6.9 m/sec *mean* $\pm$ *s.e.m.*) were consistently much greater than those in the nerve (20 ± 2.4). For the t_2 peak, the difference was smaller and more variable (tract 16 ± 3.4; nerve 13 ± 3.6), but the measured tract velocities were greater than or equal to those in the nerve in every case.

These measurements indicate that conduction velocities of both Y and X cells increase along their central course. These changes in axon diameter and velocity may be related to differences between the glial environments of the optic nerve and tract.

Supported by the Medical Research Council of the U.K.; MPS supported by the N.I.H. and the McKnight Foundation.

545.2

THE DISTRIBUTION OF IPSILATERALLY PROJECTING AXONS IN THE CAT OPTIC NERVE AND THEIR RELATIONSHIP TO PATTERNS OF FASCICULATION. G. Jeffery* (SPON: S. Judge). Dept. of Human Anatomy, Univ. of Oxford, U.K.

In order to determine the course taken by axons of known retinal origin through the optic nerve in the cat, ipsilaterally projecting axons have been traced following unilateral injections of HRP into the thalamus.

The topographic distribution of these axons changes along the length of the nerve. In the distal (behind the eye) two thirds of the nerve the majority of the labelled axons are confined to a retinotopically appropriate location, that is, laterally. However, many labelled axons are also found in central and medial regions. In the proximal third of the nerve an increasing proportion of these axons occupy the central and medial regions, so that just before the nerve enters the chiasm ipsilaterally projecting axons are found over a wide mediolateral extent. Although proximally more axons are located laterally than medially, their general distribution is relatively loose compared with that nearer the eye. The changes in the distribution of these axons along the length of the nerve are gradual.

The change in the distribution of the ipsilaterally projecting axons is in part related to a change in the organisation of the optic nerve from a fascicular pattern distally to a relatively nonfascicular pattern proximally. This change takes place gradually close to the chiasm in approximately the proximal one fifth of the nerve.

These results strongly suggest that partial retinotopic order is only present in distal regions of the cat's nerve and that the absence of retinotopic order proximally may be related to a reduction in fasciculation.

(Supported by MRC grant, no. PG 8324037)

545.3

SELECTIVE INTERRUPTION OF THE PRIMATE'S BETA CELL PATHWAY BY A SUB-CHIASMAL MENINGIOMA B.E. Reese and A. Cowey* Depts. of Human Anatomy and Experimental Psychology, Oxford OX1 3QX U.K.

There is some clinical evidence that peri-chiasmal tumours can produce functionally specific visual disturbances, for instance, impaired colour perception. The present study describes the neuroanatomical consequences of a supra-sellar meningioma in an old world monkey, in which the beta cell pathway is selectively interrupted.

This sub-chiasmal tumour compressed the nerves, chiasm and tracts along their ventral surface. Reactive gliosis was most prominent in the deeper, dorsal, half of the chiasm. The retinal ganglion cell layer of each eye showed conspicuous cell loss within the central 8mm, in all four retinal quadrants. Each optic tract showed a prominent reduction in the number of axonal profiles found deep in the optic tract, where only beta axons course. Both lateral geniculate nuclei had undergone a marked anterograde transneuronal atrophy, though confined to the parvocellular laminae, which are innervated by the axons of the beta cells. And the primary visual cortex containing the representation of the central visual field displayed a loss of cytochrome oxidase labelling in cortical layer 4A, and a reduction in the intensity of labelling in layer 4Cb, layers innervated by parvocellular geniculate neurons. These effects are considered to be the indirect consequences of the compressive lesion which has selectively affected the axons of the beta retinal ganglion cells. Their greater susceptibility may be a consequence of their size or their segregation within the chiasmal region.

545.4

MAINTENANCE OF RETINAL PROJECTIONS TO CAT DORSAL LATERAL GENICULATE NUCLEUS FOLLOWING REMOVAL OF POSTSYNAPTIC TARGET CELLS IN THE ADULT. H.E. Pearson, W.J. Sonstein* and D.J. Stoffler*. Dept. of Anatomy, Temple University School of Medicine, Philadelphia, PA 19140.

To investigate the effects of target cell removal on the survival and maintenance of afferents, kainic acid (3 nmol/ul) was injected bilaterally at multiple sites within the dLGN of adult cats. Following post-operative survivals of 2,4 and 6 months, the cats were injected intraocularly with HRP. After a further 72 hr, the cats were sacrificed and adjacent brain sections were processed for thionin staining and HRP histochemistry. Thionin staining showed large regions of dLGN to be degenerated, as characterized by the absence of neurons and increased numbers of glia. Other regions of dLGN bordering the lesion were not degenerated and contained normal cells. Sections reacted for HRP showed terminal and preterminal labelling to be present both in regions of normal cells and in regions of degeneration. HRP label was present within these regions of degeneration in all animals studied, regardless of the length of the survival period after kainic acid injection. Nuclei which contained normal and degenerated regions within the same section allowed comparison of the labelling density. At each survival time, some nuclei showed evidence for decreased density of label within the degenerated regions, whereas others did not. We conclude that retinal ganglion cells in the adult cat maintain axonal projections to dLGN upto 6 months after destruction of their postsynaptic target cells by kainic acid, and may show some capacity for rearrangement of their axonal terminals.

Supported by NS25196.

545.5

THE MAJORITY OF RETINAL CELLS PROJECTING TO THE IPSILATERAL LGBd IN THE HAMSTER ARE LARGE. G.E.Schneider, S.Jhaveri* & R.S.Erzurumlu. (SPON: K.Rockland) Dept. Brain & Cognitive Sciences, M.I.T., CAMBRIDGE, MA 02139.

Of the 3 retinal terminal types (R1,R2,R3) present in the hamster LGBd, only two types (R1,R3) are found in the ipsilateral projection zone (Erzurumlu et al.,Brain Res., *461*, 175 ['88]). None of the small R2 terminals come from ipsilaterally projecting axons. We have examined the retinal cells giving rise to this ipsilateral projection, by retrogradely labeling them from the LGBd with discrete injections of WGA-HRP, a marker reportedly not taken up by uncut axons of passage. Quantitative analysis of retinal whole mounts (cell size, number and distribution) was performed using the *Neurotrace* computer-aided microscopy system.

Our preliminary results show that **ipsilateral** to the LGBd injection, labeled cells vary in somal size from 8 µm to 18 µm (uncorrected for shrinkage - estimated at 20% for low pH, TMB-reacted tissue). Of these, **more than 60%** are larger than 13 µm and less than 10% are smaller than 10 µm. In contrast, somal sizes for labeled cells in the temporal crescent of the **contralateral** retina vary from 6 µm to 16 µm. **Less than 10%** of these are larger than 13 µm and more than 65% are smaller than 10 µm. Thus, the predominant retinal input to the ipsilateral LGBd originates in the larger ganglion cells. [Supported by NIH grants EY05504, EY00126, EY02621.]

545.6

FUNCTIONAL CHARACTERISTICS OF NICOTINIC ACETYLCHOLINE RECEPTORS IN THE RAT LATERAL GENICULATE NUCLEUS. G.T. Prusky, M.S. Cynader & R.M. Douglas Dept. of Psychology, Dalhousie University, Halifax, N.S., B3H 4J1 and Dept. of Ophthalmology, University of British Columbia, Vancouver, B.C., V5Z 3N9, Canada.

[^{3}H]Nicotine binding sites are located throughout the dorsal and ventral LGN of the rat. Removing the retinal terminals by enucleation causes a marked reduction in the density of these binding sites, indicating that many of them lie on retinal terminals. However, unlike the colliculus and the visual cortex, not all of the LGN sites are associated with terminals of extrinsic afferents, suggesting that there is another population of [^{3}H]nicotine binding sites.

Electrophysiological studies were conducted in the LGN of urethane anaesthetized rats to assess the consequences of activation of nicotine binding sites. Iontophoresis of nicotine caused a large increase in the spontaneous activity of LGN units. The activity started within 30 seconds and built steadily over the first minute. In addition, the postsynaptic components of field potentials evoked by electrical stimulation of the optic chiasm were increased in size by as much as 50%. With longer ejection durations, however, the unit firing rate gradually decreased and after 5 minutes the cells had ceased responding altogether. Responsivity to nicotine remained depressed for the next 15-20 minutes, presumably reflecting desensitization of the receptors.

These data demonstrate that functional nicotine receptors exist in the LGN and that their activation increases both spontaneous and evoked activity of LGN units.

545.7

ULTRASTRUCTURAL ANALYSIS OF THE PARABIGEMINO-GENICULATE AXONS IN THE PRIMATE

S.Feig*, D. Van Lieshout*, J.K. Harting* (SPON: T. Duff). Dept. of Anatomy, U.W. Madison, WI 53706.

The parabigeminal nucleus (PB) of Galago receives its major input from the superficial layers of the superior colliculus (SC). In addition to projecting back upon the SC, PB axons terminate within the small-celled layers of the dorsal lateral geniculate nucleus (LGN). Cells within these layers exhibit W-cell like reponses and terminate in patches within supragranular layers of area 17. We have used electron microscopic anterograde autoradiography to analyze the synaptic relationships of the PB-LGN projection. Our findings show that 50% of the silver grains lie over axon collaterals. Vesicle-filled swellings account for 25% of the labeled profiles, while synaptic terminals comprise the remaining 25%. Ninety-one percent of these synaptic terminals contain round vesicles and make asymmetrical contacts. Nine percent of the synaptic terminals contain a few pleomorphic vesicles and make symmetrical contacts. Within the small-celled layers, 75% of the PB-LGN synaptic terminals contact dendritic shafts, while 25% contact dendritic spines. Those PB-LGN terminals which contact larger dendritic shafts (the majority) lie adjacent to nonretinal terminals which contain round vesicles and make asymmetrical contacts. Preliminary data suggest that some of these nonretinal terminals arise from neurons within the SC. In contrast, when PB-LGN terminals contact smaller dendritic shafts, they are associated with retinal endings. These data suggest that nonretinal input arising from midbrain regions such as the SC and PB may play a role in modifying the relay of signals within the W-cell channel.

545.8

THE PROJECTION OF INDIVIDUAL AXONS FROM THE DORSAL RAPHE NUCLEUS TO THE VISUAL THALAMUS IN THE CAT. N. Tamamaki*, D.J. Uhlrich, J.B. Cucchiaro & S.M. Sherman (SPON: J. May). Dept. Neurobiology, SUNY, Stony Brook, NY 11794.

Ascending projections from various areas of the brainstem to the thalamus play a key role in regulating the flow of sensory information to cortex. One example is the projection from the dorsal raphe nucleus (DRN) to various visual thalamic structures. We studied the anatomical organization of this projection by injecting into the DRN of cats the anterograde tracer, *Phaseolus vulgaris* leucoagglutinin. We found labeled axons in all visual thalamic nuclei, including the ventral and dorsal divisions of the lateral geniculate nucleus (LGN_V and LGN_d), the perigeniculate nucleus (PGN) and the visual pulvinar. These axons were typically quite thin with small varicosities *en passant*. The density of labeled terminal arbors was greatest in the LGN_V; intermediate in the LGN_d C-laminae and the visual pulvinar; and very light in the LGN_d A-laminae and the PGN. DRN axons are thought to be serotonergic, and the distribution of our labeled fibers generally matches that described previously by others for serotonergic axons in the visual thalamus. From serial reconstruction, we conclude that individual axons are sparsely branched. They project across a wide retinotopic extent of the LGN_d and also to other visual thalamic nuclei. The DRN may thus globally modulate and coordinate transmission of visual signals through the thalamus.

(Supported by Japan Ministry of Education and USPHS grants EY06610 and EY03604)

545.9

SYNAPSES FROM THE PRETECTUM IN THE GENICULATE A-LAMINAE OF THE CAT. J.B. Cucchiaro, D.J. Uhlrich & S.M. Sherman. Dept. Neurobiology, SUNY, Stony Brook, NY 11794.

The pretectal nucleus of the optic tract (NOT), which seems to be involved with eye movements, receives direct retinal innervation and projects to the lateral geniculate nucleus (LGN). This may provide one route by which eye movements can influence retinogeniculate transmission. We studied further anatomical details of this pathway by iontophoretically injecting the anterograde tracer, *Phaseolus vulgaris* leucoagglutinin, into the NOT of a cat and analyzing labeled axons in the LGN at the light and electron microscopic levels. We found densely labeled axons in the geniculate A-laminae. These axons had numerous large boutons, 1.8±0.6µm in diameter, which were *en passant*. From serial electron microscopic analysis of several of these labeled boutons, we determined that they represent the sites of synaptic contacts onto geniculate cells and are strictly presynaptic. To date, all of the labeled terminals contain dark mitochondria, are densely packed with vesicles, and form symmetrical synapses. These labeled NOT terminals, therefore, have many features of F1 terminals as described by others in unlabeled material. Furthermore, most targets so far analyzed that are postsynaptic to the NOT terminals contain pleomorphic vesicles and in many ways resemble the F2 dendritic boutons of interneurons. Thus much of the influence of the NOT on the LGN may be transmitted through interneurons.

(Supported by USPHS grants EY03604 and EY06610)

545.10

TECTO-THALAMIC PROJECTIONS IN A TYPE 2 LIZARD: TOPOGRAPHY AND CELLS OF ORIGIN. N. M. Montgomery, R. Mergandahl* and K. V. Fite. Univ. Mass./Amherst

The tecto-thalamic tract of Anolis carolinensis was studied with anterograde and retrograde HRP transport. Using localized tectal injections the degree of topography in terminations was charted. Injections of HRP into n. rotundus and the dorsal and ventral lateral geniculates retrogradely labelled tectal neurons.

Tectal efferents to the pretectal nuclei and to the dorsal and ventral lateral geniculates arise from the superficial tectal layers and are topographically organized. The projection to n. rotundus is nontopographic and originates from a single population of neurons in the lower portion of layer 7. The hyperdevelopment of layer 7 is characteristic of Type 2 (iguanid) lizards.(Northcutt, 1978). Differences in tectal and pretectal cytology may be related to the tecto-rotundal system, since the latter is segregated from other ascending tectal efferents which first terminate in the central neuropil of the ventral pretectal nucleus. Axons from the ventral pretectal nucleus join those of tectal layer 7 projecting to n. rotundus and the contralateral ventral nucleus. This pattern of topographic, superficial-tectal vs. deep-tectal,nontopography occurs in R. pipiens.

545.11

TECTO-THALAMIC PROJECTIONS IN RANA PIPIENS: TOPOGRAPHY AND CELLS OF ORIGIN. K. V. Fite, N.M. Montgomery and Z. Li* Univ. of Massachusetts/Amherst

The tecto-thalamic tract was studied both with antero- and retrograde HRP transport. Topographic organization of the tract was analyzed following discrete HRP injections of tectum. Cells of origin were charted using HRP injections of thalamus.

Tecto-thalamic axons from localized tectal loci occupy different zones in the tract and have different terminations. The most superficial axons are interwoven with optic tract axons, follow the same course and project topographically to n. lentiformis mesencephali, corpus geniculatum and n. Bellonci. The rest of the tecto-thalamic tract lies medially and contains fiber bundles with different trajectories to the suprachiasmatic nucleus and posterior lateral nucleus. The most medial fibers form the tecto-bulbar and tecto-spinal tracts, which appear nontopographic.

Anterior thalamic HRP injections retrogradely labelled neurons in superficial tectal lamina 8. Posterior lateral nucleus injections labelled ganglionic cells in layer 8 and pyriform cells in layer 6. Thus, the superficial portion of the tecto-thalamic tract is topographically organized, while the medial portions originating from deep tectal laminae appear to be nontopographic.

545.12

QUANTITATIVE IMMUNOGOLD ANALYSIS REVEALS HIGH GLUTAMATE LEVELS IN AXON COLLATERAL TERMINALS OF GENICULO-CORTICAL RELAY CELLS IN THE PERIGENICULATE NUCLEUS OF THE CAT. V.M.Montero. Dept. of Neurophysiology and Dept. of Anatomy. Univ. of Wisconsin, Madison, WI 53705.

Synaptic terminals of axon collaterals of geniculo-cortical relay cells in the perigeniculate nucleus (PGN) of the cat have been identified as RLD type (Montero, Exp. Brain Res. 75:65'89) characterized by round synaptic vesicles, large size (2-6 μm) and dark mitochondria. In this study I compared in the same ultrathin section the intensity of immunogold reactivity (IGR) to a glutamate (Glu) antibody in RLD terminals in the PGN, with that of relay cell's somata (RS) and dendrites (RD), interneuron's somata (IS) and dendrites (ID), retinal (RLP), cortical (RSD) and F terminals in the lateral geniculate nucleus (LGN). In serial thin sections reacted with a GABA antibody IS, ID and F were GABA(+) while the other profiles were GABA(-). Mean densities of anti-Glu gold particles per μm^2 ± SEM in the different profiles were: RSD 30.9 ± 1.26; RLD 23.78 ± .68; RLP 22.24 ± .84; RD 17.72 ± .77; RS 16.31 ± .44; F 14.99 ± .49; IS 12.41 ± .78; ID 11.81 ± 1.01. Glu IGR differences between RLD and RLP, RS and RD, IS and ID were not significant ($p>.05$). The results (a) corroborate in cat LGN similar higher levels of Glu IGR in RSD and RLP terminals than in F terminals previously found in macaque LGN (Montero and Wenthold, Neuroscience '89); (b) show 34-46% higher Glu IGR in axon collateral terminals (RLD) than in the parent (RS,RD) geniculo-cortical neurons ($p<.0001$) suggesting higher levels of Glu associated to the transmitter pool of this amino acid in terminals vs. somata and dendrites of these cells. These immunohistochemical results are consistent with neuropharmacological data (Hagihara et al. Exp. Brain Res. 69:407'88) suggesting excitatory amino acids as neurotransmitters in the geniculo-cortical synapse.

545.13

EVIDENCE FOR TOPOGRAPHIC MAPS WITHIN THE VISUAL AND SOMATOSENSORY SECTORS OF THE THALAMIC RETICULAR NUCLEUS: A COMPARISON OF CAT AND RABBIT. J.W. Crabtree* (SPON: R.W. Guillery). Dept. of Human Anatomy, Univ. of Oxford, U.K.

Recently it has been shown that, in the visual sector of the rabbit's thalamic reticular nucleus (TRN), focal regions of visuocortical areas V1 and V2 are represented by components, or "slabs", that lie within the plane of the TRN, run parallel to its borders, and occupy only a fraction of its thickness (Crabtree, J.W. and Killackey, H.P., Europ. J. Neurosci., 1:94, 1989). In the present study of the TRN, injections of HRP and/or [3H]proline were made into visuocortical areas 17 and 18 in the cat and somatosensory cortical area SI in the cat and rabbit. The resultant anterograde labelling in the thalamus was analyzed.

In the cat, a single injection into area 17 or 18 results in a single zone of terminal label located within a dorsocaudal region of the TRN; in the cat and rabbit, a single injection into area SI results in a single zone of terminal label located in that part of the TRN lying adjacent to the ventrobasal complex. Each zone lies within the plane of the nucleus parallel to its borders and occupies a small part of its thickness. In the cat and rabbit, injections into the face representation of SI produce labelling within the inner part of the TRN, whilst those into the SI body representation produce labelling within the outer part of the nucleus.

Taken together with the previous findings in the rabbit, the present results indicate that the components representing focal regions of cortex within the TRN are similarly organized within both the visual and somatosensory sectors in both the cat and rabbit. Further, in both species there appears to be a topographic map within the TRN's somatosensory sector, one axis of which extends perpendicular to the plane of the nucleus across its thickness. A topographic map within the visual sector of the cat's TRN remains to be determined.

(Supported by the MRC, Grant No. G8720770N)

545.14

A DIRECT PROJECTION FROM THA ANTERIOR INTRALAMINAR NUCLEI TO THE STRIATE CORTEX IN KITTENS

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A direct projection of the anterior intralaminar nuclei to the striate cortex in kittens was shown by retrograde transport of HRP, Fluorogold and Rhodamine beads injected in different parts of inter and extrahemispheric area 17. The projection is ipsilateral and originates in small polymorphic neurons distributed in the three anterior intralaminar nuclei: the central medial, paracentral and central lateral. The connection has a coarse topographic arrangement as observed when displacement of the labeled area at the injection site in a caudal direction was accompained by a displacement of labeled intralaminar neurons in a rostral direction.

Complete morphology of Rhodamine labeled neurons in slices of paraformaldehyde fixed nervous tissue including the anterior intralaminar complex was seen by intracellular injection of 3-5% Lucifer Yellow. According to the distribution of dendrites, two main types of intralaminar projecting cells were observed: multipolar and inverted pyramidal-like neurons. Dendritic arborization of theese neurons were decorated with thin spines.

545.15

BURST DISCHARGES OF NEURONS OF THE LATERAL GENICULATE NUCLEUS IN CATS. S.-M. Lu, F.-S. Lo* & S.M. Sherman. Dept. of Neurobiology, SUNY, Stony Brook, NY 11794.

We recorded the extracellular responses of 40 X and 75 Y cells in the lateral geniculate nucleus (LGN) of anesthetized, paralyzed cats. Y cells often showed high frequency bursts (we define a burst as 2-5 spikes each separated by 1.2-4.0ms), while such bursts were rare in X cells. The probability of a burst in a 0.5s standard sampling period during spontaneous firing was larger for Y than X cells (15% vs. 3%, $p<0.001$), but during the 0.5s following stimulation of the optic chiasm (OX), this probability dramatically increased only for Y cells (83% for Y cells vs. 5% for X cells, $p<0.001$). The typical Y cell response to OX shock was one spike at a latency of 1.0-1.4ms, then a 100-200ms silent period preceding a burst discharge, followed by a similar silent period and burst, etc. Electrical activation of the parabrachial region (PBR) of the midbrain, which innervates the LGN, had no detectable effect on X cell responses to OX shock. However, PBR activation prior to OX shock in Y cells reduced the burst probability to the level seen with spontaneous activity (13%). Our study of 23 X and 34 Y optic tract axons revealed no such evidence of bursting, either during spontaneous activity or after OX shock. We thus conclude that this burstiness represents a response pattern during which normal retinogeniculate transmission is blocked, and active PBR input to the LGN can prevent this block.

(Supported by USPHS grants EY03038 and EY06082)

545.16

INTRACELLULAR RECORDING OF GENICULATE CELLS IN CATS: LOW THRESHOLD SPIKES. F.-S. Lo*, S.-M. Lu & S.M. Sherman. Dept. of Neurobiology, SUNY, Stony Brook, NY 11794.

From intracellular recording of geniculate X and Y cells in cats, we determined that both cell types have a voltage-dependent, low threshold spike (LTS) like the Ca^{2+}-based LTS previously described by others for thalamic neurons. This LTS is a triangular wave-form lasting 40-50ms; is activated at lower thresholds than a standard action potential; usually evokes high frequency bursts of 2-5 action potentials; is inactivated by membrane potentials above roughly -60mV; and typically requires >50ms of membrane hyperpolarization for de-inactivation. EPSPs commonly activated LTSs in Y cells, notably after stimulation of the optic chiasm (OX), when bursts recorded extracellularly are especially prevalent (see Lu et al., this volume). Activation of the midbrain parabrachial region, which effectively blocks the OX induced bursts in Y cells, reduces LTS frequency in these cells, partly, it seems, by reducing IPSP duration and thus preventing de-inactivation of the LTS. Thus the bursts we recorded extracellularly probably reflect LTSs. EPSPs rarely activated LTSs in X cells. Two possible reasons for this difference between X and Y cells are: 1) the retinal EPSP, whether spontaneous or evoked by OX shock, amply depolarizes Y cells to activate the LTS, but the X cell EPSPs are too small, although larger depolarizations of these X cells via current injection activate LTSs; 2) Y cells tend to display longer lasting IPSPs after OX shock, which may be needed to de-inactivate the LTS.

(Supported by USPHS grants EY03038 and EY06082)

545.17

TYPES OF SYNAPTIC TRIADS IN THE MONKEY LATERAL GENICULATE NUCLEUS. P. Pasik, T. Pasik and J. Hámori*. Dpts. Neurol. and Anat., Mount Sinai Sch. Med., CUNY, NY, NY 10029, and Dept. Anat., Semmelweis Univ. Med. Sch., Budapest, Hungary.

Electron microscopic observations on long series of sections of the lateral geniculate nucleus parvocellular and magnocellular laminae in monkeys (*M. mulatta*) revealed the existence of different triadic types. The intercalated element was always a GABA-containing presynaptic dendrite or soma of an interneuron (I-cell), and the output component was invariably a dendrite or soma of a geniculocortical projection or principal neuron (P-cell). The axonal input to the triads, however, could be of three types: (1) the majority were retinal axon terminals; (2) a smaller fraction were the axonal endings of corticogeniculate fibers, always connected to thin, distal P-cell dendrites; (3) still others were GABA+ terminals with pleomorphic or flattened, small synaptic vesicles, probably belonging to axons of I-cells and/or thalamic reticular nucleus neurons. In addition to these classes of triads, it was observed that the retinal terminals established multiple synaptic contacts with both P-cell and I-cell dendrites, so that two types of arrangements with retinal input were recognized: the "simple" unit, frequent in parvocellular laminae, in which the retinal axon was accompanied by only 1-2 presynaptic dendrites; and the "complex" unit, found mostly in glomeruli of the magnocellular laminae, characterized by the presence of up to eight I-cell elements. In the latter units, "closely-packed" classical triads, with the three synaptic junctions in close proximity, coexisted with triads "à distance" where the synapses were distributed relatively far from each other. The coupling of "closely-packed" and "à distance" triads by presynaptic dendrites resulted in the formation of multiple triadic arrangements. Aided by NIH Grants NS22953, NS11631 and EY01867.

545.18

SYNAPTIC INPUT TO CLASS 1/X NEURONS IN THE LATERAL GENICULATE NUCLEUS (LGN) OF THE CAT. S. C. Rapisardi, R. C. Ortiz, J. R. Wilson and A. L. Humphrey. Howard Univ., Washington D. C. 20059, and Emory Univ., Atlanta, GA 30322, and The University of Pittsburgh, Pittsburgh, PA 15261.

Analysis of synaptic input to LGN relay cells has demonstrated that retinal input to class 2/X cells is heavily involved in triadic arrangements but retinal input to class 1/Y cells is not (Wilson et al '84). However, this difference may be determined by the dominance of dendritic appendages on class 2/X neurons (Rapisardi and Miles '84). Therefore, we have studied the retinal input to X type LGN relay neurons that have relatively smooth dendrites with few appendages. Three neurons were physiologically defined as X cells and filled intracellularly with horseradish peroxidase. After analysis of consecutive thin sections, we found 23 of 54 retinal terminals involved in triads. Although this figure is intermediate to that reported by Wilson et al. for class 2/X cells (8 out of 9) and class 1/Y cells (1 out of 15), it does suggest that the morphology of the dendrite is a significant determinant of triadic involvement regardless of the physiology of the neuron. It will be necessary to perform additional detailed synaptic analysis on X and Y cells that display varying arrays of dendritic appendages before the relationship of retinal triadic involvement to the X and Y pathways can be resolved.

545.19

PHASE DIFFERENCES IN THE CAT LGN AND CORTICAL DIRECTION SELECTIVITY. A.B. Saul and A.L. Humphrey. Dept. of Neurobiology, Anatomy, and Cell Science, U. of Pittsburgh, Pittsburgh PA 15261.

Direction selectivity in visual cortex may be generated via bidirectional inputs which are in spatiotemporal quadrature (the inputs fire a quarter cycle apart in both space and time). Temporal quadrature can be obtained with conduction delays or slow synaptic potentials, but such mechanisms may work only at high temporal frequencies. Another source of temporal quadrature at low frequencies might be found in the responses of the geniculate inputs to cortex.

We characterized the spatial and temporal response properties of cells in the cat LGN. X and Y cells can be divided into lagged and non-lagged varieties. At low temporal frequencies, lagged cell response phase lags by about a quarter cycle relative to non-lagged cells of the same class and center sign. Response phase increases approximately linearly with temporal frequency, more rapidly for lagged than for non-lagged cells. Because of this, at 4-5Hz the phase difference between lagged and non-lagged cells averages a half cycle, so that temporal quadrature holds only at low frequencies.

We simulated the responses in each direction of a cell which simply sums inputs from pairs of geniculate cells. Using averaged data from lagged and non-lagged X cells, the simulation shows direction selectivity from 0.2Hz to 3Hz. At higher frequencies, the increased phase difference between the inputs causes *aliasing* (reversal of direction preference). However, lagged cells respond poorly at higher frequencies, so that little direction selectivity is actually present. In contrast, pairs of actual cells can be chosen which give strong direction preference from 0.3 to 10Hz, with little response to either direction outside this range. Pairs of non-lagged cells can be chosen which give direction selective responses at high frequencies only.

These simulations show that cortical direction selectivity can be generated by appropriate combinations of lagged and non-lagged geniculate inputs.
Supported by EY06034 and EY06459.

545.20

DIVISION OF CAT RETINAL W-CELLS INTO TWO FUNCTIONAL CLASSES BASED ON QUANTITATIVE ANALYSES OF THEIR RESPONSE PROPERTIES. M.A. McCall, A.J. Weber and L.R. Stanford. Waisman Center and Department of Comparative Biosciences, University of Wisconsin, Madison, WI 53706.

Transcleral microelectrode recordings were used to record the responses of retinal ganglion cells (n = 214) to computer-generated stationary and sine wave stimuli. Multivariate statistical analyses were then used to determine the response characteristics that could most reliably separate these neurons into discrete subpopulations. Initially, 24 response variables were entered into the analysis in an attempt to define those characteristics that best separated ganglion cells into functional subgroups. Of the 24 variables, 7 response attributes were found to account for the majority of the separation among groups. These 7 variables were used in a final cluster analysis to determine the magnitude of the distance between clusters and the mean for each variable within a cluster. Our analysis showed that quantitative analyses of ganglion cell responses can conservatively subdivide these neurons into a least 4 significantly different populations. The most powerful descriptors of the 4 functional groups were found to be axonal conduction properties and the overall level of responsiveness to visual stimuli. Two of the clusters that resulted from this analysis corresponded to the well defined retinal X- and Y-cell classes. The two remaining groups differed from X- and Y-cells primarily on their generally lower responsiveness to visual stimuli and were separable from one another on the basis of their axonal conduction properties, background discharge rates, and the duration of their response to stimuli of standing contrast. Although cells in both of these most probably belong to the heterogeneous "W-cell" classification, the members of the two groups are clearly separable on the basis of their functional characteristics.

We are currently investigating the possibility that additional response variables will further reduce the variability within the 4 clusters that we describe here and the possibility that additional quantitative data will allow us to unambiguously define further subclasses of retinal ganglion cells. Supported by NIH grant EY 04977.

545.21

EFFECTS OF BINOCULAR STIMULATION ON SPATIAL AND CONTRAST SENSITIVITY OF CAT LGN NEURONS. L. Tong, W. Guido, N. Tumosa, P.D. Spear, and S. Heidenreich. Dept. of Psychology and Center for Neuroscience, University of Wisconsin, Madison, WI 53706.

Previous studies indicate that most cat LGN neurons respond to stimulation of the nondominant eye and that the responses are spatial-frequency selective. In the present study, we examined the effects of nondominant-eye stimulation on spatial and contrast sensitivity through the dominant eye. LGN cells were tested initially for their responses to the nondominant eye alone, and the optimal spatial frequency was determined. Thirty-six cells that were inhibited and 13 cells that were excited by the nondominant eye then were tested for effects of binocular stimulation. For these tests, the dominant eye was stimulated with drifting gratings of various spatial frequencies and contrasts with or without nondominant-eye stimulation at its optimal spatial frequency. We found that nondominant-eye stimulation significantly increased or decreased response amplitude to the optimal stimulus for 23 cells. Nondominant-eye stimulation also changed the dominant-eye contrast-response function (contrast gain and/or responsivity) for many cells. However, with few exceptions, nondominant-eye stimulation had no effect on dominant-eye optimal spatial frequency or spatial resolution. A difference-of-Gaussians analysis was performed on the dominant-eye contrast-sensitivity functions of 32 cells to evaluate effects of nondominant-eye stimulation on receptive-field center and surround organization. In a small number of cells, nondominant-eye stimulation altered dominant-eye receptive-field center radius or surround sensitivity. The results thus suggest that the nondominant eye can influence LGN transmission of contrast information but has little influence on spatial-frequency selectivity for the dominant eye.

545.22

INTERACTIONS BETWEEN TWO STIMULI IN THE VISUAL FIELD: PROPERTIES OF THE CONDITIONING STIMULUS. A. Cérat*, S. Molotchnikoff. Dept. Sci. biol. and Cent. Rech. Sci. Neurol. Université de Montréal, Montréal, C.P. 6128, Canada H3C 3J7.

Recently we have reported that the introduction of a second stimulus (S2) outside the boundaries of the classical receptive field (CRF) modifies the responses of a geniculate neuron (LGN) to a stationary test stimulus (S1), (LED), positioned in the RF. The current study concentrated on the characteristics of S2. Anesthetized rabbits were prepared for single cell recordings. S2 presented alone did not modify the spontaneous firing rate. However when S2 was paired with S1 it altered the responses to S1. Results can be summarized as follows. S2 modifies primarily the secondary discharges which can be either increased (X=50.7, N = 60), or decreased (X= 35.4, N = 71). Responses increased in cells whose RF was centrally located while discharges decreased in cells with eccentric fields. A moving slit produced more profound modifications that the same stationary target (55% vs 35% p <.001 N = 19). Augmentations occured most frequently (7/8) when the movement of S2 was different to the preferred direction of the cell, while weaker responses were obtained when both movements were identical (5/9). These results indicate that the gate, represented by the geniculate neurons, is modulated by the presence of remote or "disturbing" stimuli.
Supp. FCAR and CRSNG.

546.1

LOCALIZATION OF 2-[^{125}I]-IODOMELATONIN BINDING SITES IN MAMMALIAN RETINA. C. Blazynski, C.A. Beaty*, K.C. Chung*, Z.J. Jones*, C.Woods, and M.L. Dubocovich. Dept. Ophthalmol., Washington Univ., St. Louis, MO 63110, and Dept. Pharmacol., Northwestern Univ., Chicago, IL 60611.

In retina melatonin regulates photoreceptor disc shedding and phagocytosis, melanosome aggregation in pigmented epithelium and dopamine release. The aim of this study was to localize 2-[^{125}I]-iodomelatonin binding sites in the rabbit retina using in vitro receptor binding auto-radiography. Binding sites were labeled by incubating 8 µm sections of rabbit eye cups (fixed in 4% paraformaldehyde and frozen in cryoprotectant) with 2-[^{125}I]-iodomelatonin (150 pM) in 150 mM Tris.HCl buffer (pH, 7.4) containing 0.1% BSA at 25 °C for 1 h. The highest density of specific binding defined with the melatonin receptor agonist 6-chloromelatonin (3 µM) was found (in order of magnitude) over the retinal pigmented epithelium (RPE)(52 %), inner plexiform (62 %) and inner nuclear layers (49 %), and over the outer and inner (45 %) segments of photoreceptors. The melatonin receptor antagonist, luzindole (3 µM) defined the highest density of specific binding over the inner plexiform layer (68 %) and the RPE (32 %). Thus specific 2-[^{125}I]-iodomelatonin binding sites were mainly associated with the synaptic portion of the inner retina, consistent with the role of melatonin to regulate dopamine release from dopaminergic amacrine cells in rabbit retina. Suported by grants EY-02294 to CB and MH-42922 to MLD.

546.2

REGULATION OF MELATONIN PRODUCTION IN HUMAN RETINOBLASTOMA CELLS. J.L. Janavs, M.E. Pierce*, D. Barker* and J.S. Takahashi, Department of Neurobiology & Physiology, Northwestern University, Evanston, IL 60208.

Retinas of several vertebrate species have been shown to produce melatonin in a circadian fashion. In lower vertebrates, retinal melatonin biosynthesis is stimulated by cAMP through a mechanism that requires protein synthesis. We have recently shown that Y79 human retinoblastoma cells produce melatonin in static culture. Melatonin release was enhanced by treatments that elevate cAMP. We now report that protein synthesis inhibitors block forskolin-stimulated melatonin release. Cycloheximide (10^{-6}-10^{-4}M) reduced forskolin-stimulated melatonin production by 40-80%, respectively. Anisomycin (10^{-7}-10^{-4}M) inhibited melatonin by 50-90%, respectively. To investigate whether melatonin production is a general property of human retinoblastoma tumors, we have begun to extend our studies to other retinoblastoma cell lines. We have found that static cultures of WERI Rb1 retinoblastoma cells also produce melatonin, and that release can be enhanced by forskolin, 8 Br-cAMP and the phosphodiesterase inhibitor, IBMX. Taken together, these results suggest that cAMP stimulation of melatonin production in retinoblastoma cells requires protein synthesis, as has been shown for other vertebrate retinas. Melatonin production may be a common feature of human retinoblastoma cells.

(Supported by F32 EY06167, R37 MH39592, NSF PYI DCB-8451642 and Searle Scholars 85-H-107)

546.3

MELATONIN SUPPRESSES THE LIGHT-EVOKED RELEASE OF ENDOGENOUS DOPAMINE FROM RETINAS OF FROGS (XENOPUS LAEVIS) J.H. Boatright* and P.M. Iuvone (SPON: J.R. Wilson). Emory Univ. Sch. Med., Dept. of Pharmacology, Atlanta, GA 30322.

The putative neuromodulator melatonin is considered a signal for several dark-adaptive retinal processes, including suppression of DA release. To examine the role of melatonin in regulation of DA neurons in frog retina, eye cups prepared in the last hour of the light phase from Xenopus maintained on a 12 hr light:dark cycle were dark-adapted for 90 min then incubated for 90 min under experimental conditions. DA extracted from incubation medium was analyzed by HPLC-EC and considered an index of endogenous DA release. Light-exposure of dark-adapted retinas produced a 2-4 fold increase in medium DA. Melatonin inhibited this stimulation in a concentration-dependent manner, exhibiting an EC_{50} of about 5 nM and a maximal inhibition, equivalent to that produced by incubation in darkness, at 50 nM. The putative melatonin receptor antagonists N-acetyltryptamine (NAcT), at 10 µM, and luzindole (N0774), at 5 µM, antagonized this inhibition. N0774, which was more potent than NAcT, shifted melatonin's concentration-effect curve to the right without affecting its maximal effect. However, N0774 (5 µM) did not increase DA release from eye cups incubated in darkness. These data suggest that while melatonin may play a modulatory role in the effects of lighting cues on DA release in the retina, it is not the primary mediator of these stimuli.

546.4

MELATONIN INHIBITS DOPAMINE SYNTHESIS IN AND RELEASE FROM SUPERFUSED RABBIT RETINA. B.F. Godley*, R.L. Dodd*, C. Mauron* and R.J. Wurtman (SPON: W. Nauta). Laboratory of Neuroendocrine Regulation, M.I.T., Cambridge, MA 02139.

Addition of melatonin (10^{-12} - 10^{-6}M) to the media superfusing intact rabbit retinas, suppressed the K^+-evoked (40 mM) release of endogenous dopamine (DA) in a dose dependent manner; the minimum effective concentration was 10^{-11}M. At 10^{-7}M melatonin inhibited this release maximally (by 58%, $p<0.01$). Melatonin failed to affect the spontaneous or K^+-evoked release of retinal acetylcholine (Ach). Retinal tyrosine hydroxylase (TH) activity was estimated by measuring l-DOPA accumulation following pretreatment of rabbits with NSD-1015 (100 mg/kg). Melatonin blocked K^+-induced activation of retinal TH activity by 28-48%, at concentrations as low as 10^{-11}M. DA release, measured simultaneously, was also inhibited (by 75% at 10^{-9}M, $p<0.05$). Melatonin did not inhibit TH activity in reserpine-pretreated animals. To determine whether melatonin acts directly on DA-releasing cells, we prepared dispersed retinal cells by enzymatically disrupting their synaptic connections. DA release from dispersed retinal cells was stimulated by high K^+ (40mM), and melatonin-enriched medium (100 nM) suppresed this K^+ stimulated release by 56%, ($p<0.01$). These observations show that melatonin, at low concentrations, is an effective inhibitor of endogenous retinal DA synthesis and release, and that the hormone may have a direct effect on DA-releasing amacrine neurons.

546.5

INHIBITORS OF CALCIUM ION UPTAKE IN THE RAT RETINA: QUANTITATIVE ANALYSES OF THE DOSE-EFFECT RELATIONSHIPS. J.B. Lombardini and S.M. Liebowitz. Texas Tech University Health Sciences Center, Lubbock, TX 79430 and College of Pharmacy, University of Texas, Austin, TX 78711.

Taurine is an amino acid that plays important roles in maintaining both the structural integrity and function of the retina. Thus, the effects of taurine, taurine analogues, and their combinations were studied in the ATP-dependent Ca^{++} ion uptake system at low Ca^{++} ion concentration (10 µM) in a rat retinal membrane preparation. (±)*trans*-2-Aminocyclopentanesulfonic acid (TAPS), a cyclic taurine analogue, was demonstrated to be noncompetitive (K_i=0.055 mM) with respect to taurine. 1,2,3,4-Tetrahydroquinoline-8-sulfonic acid (THQS), a less potent inhibitor of ATP-dependent Ca^{++} ion uptake than TAPS, was also shown to be noncompetitive with taurine (K_i=23.8 mM). When TAPS and THQS were tested in a fixed ratio mixture of 1:25 the inhibitory effects are synergistic as shown by the median-effect equation. (±)3-Aminotetrahydrothiophene-1,1-dioxide (ATS) and (±)piperidine-3-sulfonic acid (PSA) are an agonist and partial agonist that demonstrated stimulatory effects on ATP-dependent Ca^{++} ion uptake. ATS and taurine induced the same maximal rates of change on Ca^{++} ion uptake, however, PSA was less potent than taurine. The combination of taurine + ATS was additive while the combination of taurine + PSA was synergistic. (Supported by NIH grant EY04780 and the Welch Foundation).

546.6

EFFECTS OF SULPHUR CONTAINING EXCITATORY AMINO ACIDS AND NAAG ON ^{3}H-ACh RELEASE FROM THE RABBIT RETINA J R Cunningham and M J Neal, Division of Pharmacology, St Thomas's Hospital, London, SE1 7EH, UK

Homocysteic Acid (HCA), cysteic acid (CA), homocysteinesulphinic acid (HCSA), cysteine-sulphinic acid (CSA) and N-acetylaspartyl-glutamate (NAAG) all increased the resting release of ACh from the retina of rabbits anaesthetised with urethane. This increase in resting release was associated with a reduction in the light-evoked release of ACh. Except for NAAG, the b-wave of the erg was not significantly reduced. All five compounds were antagonised by PDA indicating that they were acting on excitatory amino acid receptors. In the rabbit retina NMDA acts as an antagonist at the bipolar/cholinergic amacrine cell synapse. NMDA antagonised the effects of HCA and HCSA but not CA, CSA, NAAG, glutamate or aspartate. Since NMDA should block exogenously added "bipolar cell transmitter" the present results suggest that of the compounds tested only HCA and HCSA are likely to be the bipolar cell transmitter(s) at cholinergic amacrine cells.

546.7

IMMUNOPROBES TO PIGMENTED EPITHELIUM OF THE RAT RETINA. L.Tien* and N.G.F. Cooper (SPON: K.U. Malik) Dept. of Anatomy and Neurobiology, The University of Tennessee, Memphis, TN 38163

Membrane proteins of retinal pigmented epithelium (RPE) may play a role in receptor mediated phagocytosis of photoreceptor outer segments (ROS). We developed immunoprobes to the RPE to explore this role, and also to determine if proteins are altered in rats with inherited retinal degeneration. Possible differences between antigens from the apical surface of RPE of normal (LE) and dystrophic (RCS) rats were analyzed by using the polyclonal antibodies. The microvilli from normal RPE were isolated by using wheat germ agglutinin (WGA) conjugated to sepharose beads. The RPE proteins were separated by polyacrylamide gel electrophoresis and Balb/c mice were immunized with three different regions (68-100kD, 100kD-200kD and >200kD) cut out from the gels. Immunocytochemical studies showed that all three antisera stained RPE cells in retina from both LE and RCS rat. The microvilli membrane proteins were assayed by Western blotting. In particular, the antiserum from mice immunized with 68-100k range of membrane proteins recognized two proteins with molecular weight 186K and 100k from both LE and RCS rats. No significant differences were detected between normal and dystrophic proteins with this antiserum. With immunohistofluoroscence of aldehyde fixed tissue-sections, this polyclonal antibody also showed a bright band of staining at the junction of the neural retina with RPE and there was a lack of immunostaining in the inner retina. Immunoelectron microscpy showed that the antigen was present on the apical surface of the RPE and was associated with the microvilli.

Supported by NIH:NEI EY02708(NGFC).

546.8

LIQUID COLUMN PURIFICATION OF RETINYL ESTER HYDROLASE IN THE NEURORETINA. A.T.C.Tsin and D.W. Malsbury* Division of Life Sciences, The University of Texas at San Antonio, San Antonio, TX 78285.

Retinyl ester hydrolase is an enzyme which catalyzes the hydrolysis of retinyl esters into retinol and fatty acids. Recent reports show that retinyl ester hydrolase is located in the neuroretina as well as in the pigment epithelium of the eye (Rodriguez, K.A. and Tsin, A.T.C., Amer. J. Physiol. 256:R255-R258, 1989). In the present study, retinyl ester hydrolase was purified to homogeneity employing a sequence of three liquid columns. One hundred bovine retinas were homogenized in 600 ml of 10 mM Tris-Maleate buffer, pH 8 and the soluble fraction (after centrifugation at 100,000 g for 30 min) was introduced onto a DEAE-cellulose column (7 X 25 cm; flow rate: 2 ml/min.; 2000 ml linear gradient of 0 - 1 M NaCl in 0.01M Tris-Maleate, pH 8). Fractions containing retinyl ester hydrolase activity were pooled and concentrated to 2 ml and introduced into a Sephadex G-200 column (1.6 X 70 cm; flow rate: 0.1 ml/min.; eluted with 150 mM phosphate buffer, pH 7). REH was finally purified to homogeneity by HPLC (high performance liquid chromatography; Protein Pak 300 SW, 8 mm X 30 cm, flow rate: 1 ml/min.; eluted with 0.15 M NaCl). REH activity towards 11-cis and all-trans substrates co-eluted in all three columns in the present study. The specific activity of the purified REH was about 1000 fold higher than that of the homogenate. Pure REH has a single protein band on SDS-PAGE (sodium dodocyl sulfate - polyacrylamine gel electrophoresis) and a molecular weight of 75,000 Dalton. Supported by grants from the NIH, DoD and the San Antonio Area Foundation.

546.9

TRYPTOPHAN HYDROXYLASE ACTIVITY IN CHICKEN RETINA AND PINEAL GLAND DISPLAYS CIRCADIAN RHYTHMICITY. K.B. Thomas* and P.M. Iuvone (Spon: J.W. Manning). Dept. of Pharmacology, Emory Univ. Sch. of Med., Atlanta, GA 30322.

Tryptophan hydroxylase is the rate-limiting enzyme in the biosynthesis of serotonin, a neuromodulator and precursor of melatonin. Our studies on tryptophan hydroxylase in the retina and pineal of chickens suggest that the enzyme is regulated by circadian oscillators. Accumulation of 5-hydroxytryptophan, the tryptophan hydroxylase reaction product, after inhibition of its metabolism to serotonin, was measured as an index of in situ tryptophan hydroxylase activity. For animals maintained on a 12 h dark: 12 h light cycle, greatest activity was observed 4 hours into dark phase; minimum activity occurred 4-8 hours into light phase. A 3 - 5 fold light-dark difference in activity was observed in retina, 2 fold in pineal. Onset of increased activity precedes the beginning of dark phase. The D2 agonist quinpirole inhibits the nocturnal increase of retinal tryptophan hydroxylation, suggesting a dopaminergic component in the regulatory mechanism associated with the rhythmic changes in activity. As melatonin levels fluctuate in a similar circadian fashion and are regulated in retina by dopamine, regulation at the tryptophan hydroxylase step may contribute to the regulation of melatonin biosynthesis.

546.10

CHARACTERIZATION OF TAURINE RECEPTORS IN RETINAL PIGMENT EPITHELIUM CELLS IN CULTURE. A.M. López-Colomé, R.Salceda and G. Fragoso. Instituto de Fisiología Celular, UNAM. Apdo. Postal 70-600, 04510 México, D.F.

Taurine is highly concentrated in the retinal pigment epithelium (RPE), and a high affinity uptake system for this compound has been characterized in several species. Taurine is also concentrated in the retina, particularly in rod outer segments (ROS) from which it is released by illumination. A close interaction between RPE and the retina has been demonstrated in phenomena as ROS shedding and in the ionic regulation of the subretinal space. Since an action of taurine on RPE should be mediated by a receptor interaction, we here explored the existence of specific taurine receptors in membranes from primary cultures of RPE. Cell membranes were obtained following the classical procedures, and ^{3}H-taurine binding was measured. Saturable, high-affinity binding of taurine was detected, with K_D = 237 nM and B_{max} =2.8 pmole/mg protein. Binding showed to be Na^+-independent, higher at 37°C compared to 4°C, and inhibited most potently by glycine, followed by strychnine, bicuculline, GABA and β-alanine. Binding was higher (specific = 50%) in frozen than in freshly obtained membranes, and similar at days 16 and 25 in vitro. Since these binding sites do not seem to correspond to uptake sites, it could be suggested that taurine could act as a messenger from the retina to the RPE through an interaction with the receptors we have described.

546.11

AN IMMORTALIZED LINE OF RETINAL NEURONS DERIVED FROM PNMT-SV40 TRANSGENIC MICE. J.P.Hammang, E.E.Baetge, R.R.Behringer*, E.P.Sandgren*, R.D.Palmiter*, R.L.Brinster*, and A.Messing. Sch. of Vet.Med., Univ. of Wisc., Madison, WI 53706; Bristol-Myers, Wallingford, CT 06492; Sch. of Vet.Med., Univ. of Penn., Philadelphia, PA 19104; H.H.M.I., Univ. of Wash., Seattle, WA 98195.

SV40 T antigen expression was directed to adrenergic cells of the adrenal gland and retina (amacrine neurons) of transgenic mice using 5' flanking sequences of the human PNMT gene. Retinal tumors were induced and tumor-derived cells have been propagated in tissue culture for over 68 passages. The cells have a doubling time of ~24 hours and spontaneously display a neuronal morphology with extensive neuritic processes. ~95% of the cells are T antigen positive by immunofluorescence. Time-lapse cinematography of the neurite-bearing cells revealed that the neurites are retracted prior to cell division and extended again 3-4 hours after cytokinesis. We identified the cells as either amacrine or horizontal neurons using immunocytochemical markers (A2B5 and VC1.1 [Arimatsu et al., J.Neurosci., 7:1250, 1987]). The retinal cells do not exhibit the adrenergic phenotype at the level of PNMT mRNA, protein, or catecholamine histofluorescence. A second retinal cell line is currently being characterized utilizing a temperature-sensitive T antigen in which more complete differentiation may be possible at the restrictive temperature.

546.12

SEROTONIN N-ACETYLTRANSFERASE ACTIVITY IN PHOTORECEPTOR-ENRICHED RETINAL CELL CULTURES: ROLES OF CYCLIC AMP, Ca^{2+} AND DOPAMINE. P.M. Iuvone and G.A. Avendano*. Dept. of Pharmacology, Emory Univ. Sch. of Med., Atlanta, GA 30322.

Regulation of serotonin N-acetyltransferase (NAT), a key regulatory enzyme in melatonin biosynthesis, was studied in glia-free, low-density retinal cell cultures prepared from embryonic day 6 chicks. Photoreceptors represented approximately 70% of the cells in these cultures; the remaining cells were multipolar neurons and apparently undifferentiated round cells. NAT activity in these cells was markedly stimulated by agents that increase intracellular cyclic AMP, such as forskolin, isobutylmethylxanthine, and 8-Br-cyclic AMP. Stimulation was blocked by inhibitors of RNA and protein synthesis. Elevated K^+ also increases NAT activity by a mechanism that involves Ca^{2+} influx through dihydropyridine-sensitive voltage-gated channels, as the K^+-evoked increase was blocked by nifedipine and potentiated by BAY K 8644. Elevated K^+ also increased intracellular cyclic AMP in the cells, suggesting a possible mechanism for the effect on NAT activity.

Dopamine inhibited the increases of NAT activity elicited by forskolin and by K^+. The effect of dopamine was blocked by the D_2-dopamine receptor antagonist sulpiride or by pretreatment with pertussis toxin. Dopamine also decreases K^+ stimulated cyclic AMP levels. Thus, dopamine appears to regulate NAT activity in these cell cultures through a D_2-dopamine receptor - G protein - adenylate cyclase complex.

546.13

TRANSFERRIN BINDING IN EMBRYONIC NEURAL RETINAL CULTURES. A.G. Hyndman. Dept. of Biological Sciences, Rutgers University, Piscataway, NJ 08855.

Transferrin may be important in the regulation of neural differentiation. In order to increase our understanding of transferrin's role in development, high affinity (pmol range) transferrin (tf) binding in purified neuronal cultures from both E8 and E11 chick neural retinas was examined. TF binding reaches a plateau in 30 min at 4° C and 2 hr at 37° C. The analysis of tf high affinity binding sites at 37° C demonstrated the following: 1) tf binding in E8 cultures was 2-fold greater than in E11 cultures, 2) binding activity can be increased by maintaining neurons in the presence of tf. Maintaining cultures in the presence of 20nM tf increased tf high affinity binding by 45% and 80% in cultured neurons from E8 and E11 respectively. Transferrin increased the maximal number of high affinity binding sites at both ages, suggesting that transferrin can "up regulate" these receptors. There was little difference in the affinity (Kd) of these binding sites in various conditions tested, except in E8 cultures maintained in the presence of tf. In this case, tf affinity increased suggesting that the tf stimulation of high affinity tf binding sites in E8 cultures may result in the increased appearance of immature sites which are less effective at tf binding. Cultures maintained in the presence of 0.2mM free iron increased tf binding in E8 cultures by approximate 25% and by 20% in E11 cultures. Transferrin binding at 4° C gave similar results. NSF BNS-8810277.

546.14

MEMBRANE CURRENTS OF XENOPUS LAEVIS OOCYTES INJECTED WITH RNA FROM RETINAS OF CARP AND MOUSE. Lawrence H. Pinto, Neurobiology & Physiology Dept., Northwestern University, Evanston, IL 60208. Akimichi Kaneko, National Institute for Physiological Sciences, Myodaiji, Okazaki, 444 Japan. Cathy Bowes and Deborah Farber, Jules Stein Institute, UCLA, Los Angeles, CA 90024-1771.

We measured voltage-clamp currents 3-7 days after injection of total RNA from retinas of adult carp (N=24 oocytes) or 9-11 day old C57BL/6J mice (N=34). Controls were uninjected (N=40). After injecting carp RNA (0.7 mM $[Ca^{2+}]o$) depolarization (holding voltage, Vh, of -90mV) produced an outward current that was attenuated by 20mM TEA, 1 mM Co^{2+}, or 500 μM DIDS. Thus, $I_{K(V)}$, I_{Ca} and $I_{Cl(Ca)}$ flowed in these oocytes. For oocytes injected with mouse RNA, depolarization (Vh=-90 mv) also produced a DIDS-sensitive outward current, but in 40 mM barium methanesulfonate the current became inward for depolarization to -10 to +20 mV. An inward tail current flowed after offset of the depolarizing pulse with 0.7 mM $[Ca^{2+}]_o$; this current was larger for oocytes injected with mouse RNA than for uninjected oocytes. The amplitude of the tail current was increased by increasing $[Ca^{2+}]_o$, and was decreased by substitution of Ba^{2+} or Co^{2+} for Ca^{2+} or addition of 400 μM DIDS. No TTX-sensitive currents were observed. Thus, I_{Ca} and $I_{Cl(Ca)}$ of larger amplitude flowed in oocytes injected with mouse retinal RNA than flowed in uninjected oocytes.

VISUAL CORTEX VII

547.1

INTERLAMINAR DISTRIBUTION OF MUSCARINIC ACH RECEPTORS IN STRIATE CORTEX. B. N. Baker*, K. K. Glendenning and R. B. Masterton (SPON: D. M. Easton). Dept. Psychology, Florida State Univ., Tallahassee, FL 32306.

Conventional quantitative labeling of muscarinic ACh receptors in visual cortex with tritiated QNB was compared in 7 species of mammals: 2 marsupials and 5 placentals, including 2 species of prosimian primates.

The results show that the density of mACh receptors in striate cortex can vary as much as 5 to 1 among species. However, more than 97% of this variation can be accounted for by 3 visual parameters: type of retina, vision-dominance, and diurnal cycle. Furthermore, these same 3 parameters account for 95% of the variation in receptor density in layer 4 and in the infragranular layers and for 97% in the supragranular layers.

However, when the density of receptors in the supragranular layer is normalized in each animal by using its ratio with receptor density in layer 4 (i.e., SG/4), the three vision parameters lose their explanatory power, now accounting for less than 20% of the variation. Instead, "relative degree of kinship with Anthropoids" or "phyletic grade" accounts best for the remaining variation with a single sharp increase in supragranular density occurring between the marsupials and placentals regardless of whether the placentals are Primates and regardless of the other variations in morphological form of their visual system. Supported by NIH-NINCDS # NS7726.

547.2

THE LECTIN VVA LABELS A MORPHOLOGICALLY HETEROGENEOUS SUBPOPULATION OF GABA NEURONS IN THE MONKEY STRIATE CORTEX. K.A. Mulligan & A.E. Hendrickson. Dept. Biological Structure and Ophthalmology, Univ. of Washington, Seattle, WA 98195.

In the cerebral cortex of several species the lectin from *Vicia villosa* (VVA) labels the surface of a subpopulation of GABA cells. In monkey striate cortex, VVA labels about 30% of all GABA cells (Mulligan et al, '89, Vis. Neurosci. 2:63). Although the largest GABA cells in each layer label with VVA, it is unlikely that the labeled cells constitute a single cell type. In this study we further characterize the VVA-labeled cells by double-labeling experiments and by intracellular injection of VVA-labeled cells in fixed slices of macaque striate cortex.

Single section double-labeling experiments using different colored chromogens showed that virtually all VVA-labeled cells were immunonegative when reacted with antisera to known neuropeptides and to the calcium-binding protein, calbindin. However, nearly all VVA-labeled cells contained parvalbumin, another calcium-binding protein.

Intracellular injection of Lucifer Yellow into VVA-labeled cells revealed at least three clear patterns of dendritic morphology. Large, multipolar cells with widely spreading varicose dendrites were recovered from injections in layers 5 and 6. In layers 2 to 4 small multipolar cells with smooth radial dendrites were recovered, while injections in layers 2 and 3 often revealed medium-sized cells with prominently vertical dendrites. Thus, although the population of VVA-labeled cells appears neurochemically homogeneous, distinct morphological subtypes exist. Supported by NIH grants EY01208 and EY04536.

547.3

GABA and the synaptic or non-synaptic localization of benzodiazepine/$GABA_A$receptor/Cl^-channel complex in visual cortex of cat. P. Somogyi[*1], J.D.B.Roberts[*1], A.Gulyas[*1], J.G. Richards[2], A. L. De Blas[3] (SPON:A. De Blas) [1]MRC Anat. Neuropharm. Unit, S. Parks Rd, Oxford,U.K., [2]Pharm. Res. Dept, Hoffmann-La Roche. Basle, Switzerland, [3]Dept. Neurobiol. State Univ. New York. Stony Brook.

The subcellular distribution of the receptor complex was determined using monoclonal antibodies to either the α- or the β-subunit and EM immunocytochemistry. Both subunits were distributed similarly. Intracellular immunoreactivity was associated with the endoplasmic ret., Golgi system and multivesicular bodies, suggesting the synthesis, glycosilation and degradation of the receptor. Extracellular immunoreactivity was on the membrane of neuronal somata, dendrites and spines. Immunoreactivity appears to be uniform at presumed GABAergic synaptic junctions and at the non-junctional plasma membrane.

Immunoreactivity is highest in layer 4. Different classes of neuron express the receptor complex to different degrees. Some GABA-containing cells, especially the large basket cells, show the highest density of receptor on the plasma membrane of their somata and dendrites. Some GABA-negative cells show very strong intracellular reactivity suggesting a high turnover of the protein. The results predict differential sensitivity of different neuronal classes to $GABA_A$ agonists, which could act both at synaptic and non-synaptic receptor sites.

547.4

DOWN-REGULATION OF $GABA_A$ RECEPTORS IN AREA 17 OF MONOCULARLY DEPRIVED ADULT MONKEYS. S.H.C. Hendry, J.L. Fuchs, A.L. deBlas and E.G. Jones. Dept. of Anatomy & Neurobiology, University of California, Irvine, CA 92717, Dept. of Biological Sciences, Univ. of North Texas, Denton, TX 76203 and Dept. of Neurobiology & Behavior, SUNY, Stony Brook, NY 11794.

Within ocular dominance columns driven by a deprived eye, the number of neurons immunoreactive for GABA is reduced by one-half (Hendry and Jones, 1988, Neuron 1:701). We used immunocytochemical and radioligand binding methods to determine if receptors for GABA are similarly affected. The distribution of $GABA_A$ receptors was examined in adult macaques (M. fascicularis and M. mulatta) that were normal, had one eye removed or had tetrodotoxin (TTX) injected into one eye. The highest density of receptors immunostained with a monoclonal antibody to the $GABA_A$ complex in normal animals was in layers IVCB, IVA and II-III. Layer IVCB was uniformly stained in normal monkeys. A similar laminar distribution was seen in autoradiograms of ^{3}H-muscimol and ^{3}H-flunitrazepam binding where the radiolabeling in layer IVCB was also uniform. Following eye removal or TTX injections, stripes of normal immunostaining or radiolabeling in layer IVCB alternate with stripes of reduced staining or labeling. Comparison with adjacent sections stained for cytochrome oxidase showed the normal staining/labeling to be in normal-eye dominance columns and the reduced staining/labeling to be in deprived-eye columns. Quantitative autoradiography showed the density of receptors was reduced by 25-30% in layer IVCB of the deprived-eye columns. These data demonstrate that parallel reductions in GABA and $GABA_A$ receptors occur in deprived-eye columns, suggesting that GABA transmission is impaired in the deprived adult monkey visual cortex.

Supported by NEI grants EY07193 and EY06432.

547.5

THE CALCIUM-BINDING PROTEIN PARVALBUMIN IN THE STRIATE CORTEX OF MACAQUE MONKEYS AND HUMANS.
I. Bluemcke~, P.R. Hof, J.H. Morrison and M.R. Celio. Anatomy Dept., University of Kiel, 2300 Kiel, F.R.G. and Preclinical Neuroscience of Scripps Clinic, La Jolla, CA 92037.

We analysed the occurrence of parvalbumin (PV) in the primary visual cortex (V1) in both primates to detect similarities and differences in the organisation of V1. In both primates the distribution of parvalbumin - immunoreactive (PV-ir) neurons and terminal fields matches that seen with cytochrome C-oxidase. However, the band-like PV-ir terminal fields of layer 4A are missing in human. Layer 1 is devoid of PV-ir cells in both species and we find a lower amount of neurons in human layer 2. Morphologically PV-ir neurons resemble smooth dendritic multipolar-bitufted- and stellate cells. In human V1 we find larger cells in layers 4B, 4Cα, 5 and 6. Immunoelectron-microscopy of monkey V1 reveals many PV-ir synapses of the symmetrical, but also some of the asymmetrical type. The white matter contains PV-ir axons, located in efferent and afferent pathways. We assume that PV supports crucial functions of highly specialized neurons during visual perception.

547.6

INTRINSIC CONNECTIONS OF CYTOCHROME OXIDASE (CO) BLOB AND NONBLOB REGIONS IN AREA 17 OF A NOCTURNAL PRIMATE.
V.A. Casagrande, P.D. Beck, and E.A. Lachica**. Dept. Cell Biol., Vanderbilt Univ., Nashville, TN 37232.

The combination of laminar location, connections, and the pattern of CO activity in macaque monkey area 17 are considered useful markers of three functional channels. In layer III, CO blob and non-blob zones are thought to be the indirect targets of information from the parvo-cellular LGN layers and represent, respectively, one channel for color perception and a second channel for perception of form. Layer IVB, known to be the indirect recipient of magnocellular LGN input, is thought to represent a third visual pathway for motion perception. We examined the generality of this view by injecting HRP into blob and non-blob zones in the nocturnal ***Galago***, a primate with well developed blobs, few, if any, retinal cones, and no striate layer IVB. HRP injections within blobs labelled cells retrogradely in both layers IV***β*** ***and*** IV***α*** (respective targets of parvo- and magnocellular LGN layers) as well as layers V and VI. Injections into either nonblob zones or layer IIIC (whose cells project to motion area MT) labelled cells ***only*** in layers IV***α***, V and VI. Thus, by analogy to work in macaque, blobs in galago may carry information about form, while both nonblob zones and layer IIIC may carry information that may be important for motion perception. (Supported by EY01778 to VAC & MH09754 to EAL).

547.7

EFFERENT PROJECTIONS OF LAYER 4 IN TREE SHREW STRIATE CORTEX: EVIDENCE FOR PARALLEL PATHWAYS TO THE SUPERFICIAL LAYERS.
E. Muly, D. Fitzpatrick, and D. Raczkowski, Dept. of Neurobiology, Duke Univ. Med. Ctr., Durham NC, 27710.

Layer 4 in tree shrew striate cortex is divided into two tiers separated by a cell sparse cleft. The upper tier, 4A, receives projections from ON-center LGN neurons; the lower tier, 4B, receives projections from OFF-center neurons. We studied the projections of 4A and 4B using micro-injections of HRP and WGA-HRP.

Our results show that each tier consists of two parts that differ in their pattern of projection to the superficial layers. Injections in the upper part of 4A or lower 4B label axons and terminal arbors in layer 3C. In contrast, injections in lower 4A or upper 4B label bundles of axons that pass through 4A and 3C to terminate in layer 3B. These anterograde patterns are consistent with patterns of retrograde transport. Injections in layer 3B label cells immediately adjacent to the cleft, while injections limited to 3C label cell bodies in upper 4A and lower 4B.

We conclude that two distinct and parallel paths convey ON and OFF information from layer 4 to layers 3B and 3C. Since the central region of layer 4 receives input from the contralateral eye, while the flanking regions receive binocular input, layers 3B and 3C may differ in the way they process the inputs from the two eyes. Supported by NIH grant EY06821.

547.8

LAMINAR DISTRIBUTION AND MORPHOLOGY OF AREA 17 NEURONS PROJECTING TO THE LATERAL GENICULATE NUCLEUS IN THE MACAQUE.
D. Fitzpatrick and G. Einstein. Dept. of Neurobiology, Duke Univ. Med. Ctr., Durham, NC 27710.

It has been suggested that the corticogeniculate (CG) pathway of the primate comprises two populations of layer 6 neurons, one projecting to the magnocellular layers and another to the parvocellular (Lund et al.,'75). We have reexamined the organization of this pathway in the cynomolgous monkey using retrograde tracing methods in combination with intracellular dye injections in fixed cortical slices (Einstein, '88). Injections of retrograde tracers involving both the magno- and parvocellular layers reveal two distinct tiers of labeled neurons within layer 6: one tier at the top, and another at the bottom separated by a middle zone largely free of labeled neurons. Restricted injections suggest that the upper tier projects only to the parvocellular layers while the lower tier projects to both magno- and parvocellular layers. Intracellularly injected CG neurons in the upper tier have pyramidal-shaped cell bodies. Their basilar dendrites descend into the middle zone and their apical dendrites extend to more superficial layers with branches in layer 5. In contrast, many CG neurons in the lower tier have ovoid-shaped cell bodies with extensive dendritic arbors in that tier. Taken together, these results support the existence of distinct streams within the CG pathway. Supported by NIH grants EY06661 and EY07840.

547.9

TRANSCALLOSALLY PROJECTING NEURONS IN CAT VISUAL CORTEX.
Alan Peters and Bertram Payne. Department of Anatomy, Boston University Medical School, Boston, MA 02118.

A large number of neurons that had been filled following injection of HRP into the contralateral marginal gyrus were examined by light microscopy. The purpose was to find reactive neurons that did not have the features of either pyramidal or spiney stellate cells, to ascertain whether inhibitory neurons project axons across the corpus callosum. Of several hundred neurons displaying a Golgi-like filling, five neurons that appeared to lack the features of spiney neurons were selected for electron microscopic evaluation. All but one of these cells was revealed to be a poorly filled spiney neuron. The remaining neuron was a non-pyramidal cell from layer IV. It was a large cell with stout dendrites forming a spherical dendritic tree. The nucleus had a folded envelope and the soma had both symmetric and asymmetric synapses, typical of a non-pyramidal cell; perhaps a large basket cell. Next, material containing over 100 transcallosally projecting neurons reacted using the more sensitive TMB or TMB-DAB technique were examined in 2μm thick plastic sections and adjacent thin sections. None of these cells proved to have the features of non-pyramidal cells with both symmetric and asymmetric axosomatic synapses. It would appear then, that although non-pyramidal cells can project across the corpus callosum, their number is few, and not proportional to the number of GABA containing neurons (about 15-20%) in cat visual cortex. It will be argued, however, that this conclusion may be incorrect, for there is evidence that although non-pyramidal cells do transport HRP across the corpus callosum, the amount is too small to permit a diffuse labelling of the kind normally accepted as representing a positive reaction. (Supported by EY06404)

547.10

INTRATELENCEPHALIC CONNECTIONS BETWEEN THE VISUAL AREAS IN BIRDS (*Columba livia*).
T. Shimizu, W. Woodson*, H. J. Karten and J. B. Schimke*. Dept. of Neurosciences, Univ. of California, San Diego, La Jolla, CA 92093.

The two major visual pathways to the telencephalon in the avian brain are the thalamofugal pathway (geniculo-striate pathway of mammals) and the tectofugal pathway (tecto-thalamo-extrastriate pathway of mammals). The visual wulst is the primary telencephalic target of the thalamofugal pathway, and contains four distinct laminae (HA, IHA, HIS and HD). The telencephalic recipient of the tectofugal pathway is a non-laminated aggregate, the core region of the ectostriatum (Ec). Horseradish peroxidase (HRP) and Phaseolus vulgaris Leucoagglutinin (PHA-L) were used to study interconnections of these two visual areas. Efferents of Ec were mainly observed in the surrounding peri-ectostriatal belt (Ep) with a few projections to the neostriatum. The Ep projects to the lateral neostriatum intermedium (NIL), confirming the report of Ritchie and Cohen (1977). When HRP was injected into the Ec, retrogradely labelled cells were found in the anterior lateral portion of the hyperstriatum ventrale (HVal). Using PHA-L, the HD/HIS of the visual wulst was found to project heavily to the HA, and moderately to the lateral neostriatum frontale (NFL), Ep, and HVal. These results suggest 1) multiple processing streams from the striate to the extrastriate areas in non-mammals, 2) convergence of information from the two pathways in the extrastriate area (Ep), and 3) intratelencephalic connections upon the extrastriate system similar to mammals, despite the lack of a laminar configuration. (Supported by ONR N00014-88-K-0504 and NINCDS PHS NS24560-03).

547.11

Morphological features of Area 17 efferents to extrastriate cortex in the tree shrew (*Tupaia belangeri*).

M. A. Sesma and P. Meyer, School of Optometry, University of Missouri - St. Louis, 63121, U.S.A.

We have been examining features of visual cortical connections in the tree shrew using a variety of anatomical methods. In the tree shrew, Area 17 has major projections to two extrastriate cortical areas, area 18 and the temporal dorsal area (TD). These projections are multiple and periodic and may arise from different populations of neurons in the supragranular layers of area 17. To evaluate the morphology of individual efferent axons and the cells of origin in area 17 we used injections of the lectin, phaseolus vulgaris leucoagglutinin (PHA-L) or WGA-HRP.

Electrophoretic injections of PHA-L or pressure injections of WGA-HRP were made in area 17 of anesthetized tree shrews and post-injection survival ranged from 1 (WGA-HRP) to 10 days (PHA-L).

Reconstructed axons projecting to area 18 and TD had long, relatively straight trajectories with little or no branching until arborizing at discrete sites. Axonal arborizations in area 18 and TD were distributed over the middle layers with a horizontal extent of 350-400μm. Some axons had several arbors separated by gaps of 200μm-300μm but were restricted to a single area. In both areas the arborizations had en passage boutons and clusters of terminal boutons. These axons originate from small to medium size pyramidal cells in supragranular layers of area 17. Those cells projecting to area 18 lie throughout the supragranular layers while those projecting to area TD are restricted to layers II-IIIa.

These studies indicate that neurons in area 17 possess axons that arborize within several distinct zones in a single extrastriate area. It is likely that area 17 conveys different information to area 18 and area TD.

Supported by NSF Grant BNS:8618448 and an Improved Research Quality Grant from the University of Missouri-St. Louis to M.A.S.

547.12

SYNAPTIC ORGANIZATION OF CORTICO-CORTICAL CONNECTIONS IN THE VISUAL CORTEX OF THE CAT. P.R. Lowenstein and P. Somogyi*. MRC Anatomical Neuropharmacology Unit, Department of Pharmacology, South Parks Rd., Oxford, OX1 3QT.

The particular response properties of neurons in the Clare-Bishop area probably arise through an interaction of the thalamic input with afferents from the striate cortex or intrinsic cortical circuitry. In order to understand the neuronal circuits subserving the physiological characteristics of cells in this area, we have studied the synaptic organization of the inputs from area 17 onto pyramidal and GABAergic neurons in the suprasylvian cortex of the cat. The anterograde tracer PHA-L was delivered iontophoretically into area 17 and the transported lectin was revealed by immunohistochemistry. A postembedding EM immunogold method for the detection of GABA was utilized to establish to what extent GABAergic inhibitory neurons receive direct cortico-cortical input. As previously described we found most of the terminals from area 17 in layer IV. The majority of postsynaptic targets were dendritic spines (75%), probably belonging to pyramidal cells, while 20% were dendritic shafts and 5% of the targets could not be identified. The majority of dendritic shafts tested so far were GABA positive, and therefore belong to inhibitory neurons. Our results show that cortico-cortical terminals mainly innervate pyramidal neurons and to a lesser extent GABAergic cells in the suprasylvian cortex.

547.13

SUBCORTICAL CONNECTIONS OF VISUAL AREAS MST AND FST IN MACAQUES. Driss Boussaoud*, Leslie G. Ungerleider, and Robert Desimone. Lab. Neuropsychology, NIMH, Bethesda, MD 20892.

Visual areas MST and FST, in the dorsal bank and floor, respectively, of the caudal superior temporal sulcus both receive direct projections from area MT, which is known to be specialized for motion analysis. Most MST and FST cells are directionally selective, and many cells in MST respond during pursuit eye movements (Newsome et al., '88). In the present study, we investigated the subcortical connections of MST and FST and compared them to those previously reported for MT (Ungerleider et al., '84) by injecting the two areas with retrograde and anterograde tracers in 7 cynomolgus monkeys.

MST and FST project to the pulvinar complex, claustrum, striatum, reticular nucleus of the thalamus, superior colliculus, and pontine nuclei. Both areas, in turn, receive projections from the pulvinar and claustrum. The connections with the pulvinar involve the medial, lateral, and inferior nuclei, while those with the claustrum involve the ventral portion. The projections to the striatum terminate posteriorly, in the genu of the caudate and in the ventral putamen. Projections to the superior colliculus terminate in the upper and lower layers of the stratum griseum superficiale. Projections to the pontine nuclei terminate rostrally in the lateral, dorsolateral, dorsal, and dorsomedial nuclei. Finally, in a few instances, we found projections from the lateral posterior nucleus of the thalamus to MST and from the basal forebrain to FST. The results indicate that while subcortical projections of MST and FST in the macaque overlap considerably with those of MT, those from MST and FST are more extensive to the pons, consistent with the role of these areas in visuomotor function.